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Potential Environmental Impact of Widespread Releases of Non-Ice Nucleating Bacteria in Agriculture

Report to the Office of Technology Assessment,
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Executive Summary

We analyse the potential effects on precipitation of widespread agricultural release of ice-nucleation-activity-minus (INA^-) bacteria. The structure of our analysis follows the process of invasion and spread in new habitats. We determine the cropland area that INA^- bacteria could be applied, examine the interactions of INA and INA^- bacteria on the phylloplane, model the spread of INA^- bacteria, and consider the impact of reduced INA bacterial populations on precipitation patterns. The analysis is complicated by the multiple spatial and temporal scales under consideration and the relative dearth of knowledge about most of the details of the process.

We complete three analyses. First we develop an absolute worst case analysis, where few restrictive assumptions are made. We conclude that the possibility of climatic impact cannot be summarily dismissed as out of hand, so we develop a biologically constrained scenario. In this scenario, we analyse a worst case and a more probable case. Our conclusions for these cases follow.

Conclusions

1. Widespread release of INA^- bacteria in agriculture is not likely to cause long-term or global effects on precipitation.

Release rates are not likely to be very high. Possible rates, including subsequent reproduction, are 10^{-2} to 10^{-5} of the total bacterial population. Probable rates are nearer 10^{-5}. These probable rates derive from 1) not all of the cropland planted to frost sensitive crops is frost vulnerable, 2) it will not be profitable to use INA^- bacteria on all frost vulnerable cropland, and 3) INA$^-$ bacteria will not be used in all cases even if it is more profitable.

In the probable case, spread will occur very slowly, taking thousands of years and the probability that INA bacteria would be eliminated is very small, less than 0.005.

In the worst case, spread could occur quickly, taking about 100 years, and the probability that INA bacteria would be eliminated is very high, 0.39. The most critical assumption is that inter-strain competition is greater than intra-strain competition. If this were not true, then the probability that INA bacteria would be eliminated by INA$^-$ bacteria is near zero.

2. Widespread release of INA$^-$ bacteria in agriculture could cause significant local or short-term effects on precipitation.

Local displacement of INA bacteria can occur if enough INA$^-$ bacteria are released early enough in the growing season. Theory predicts that these populations could exclude large populations of INA bacateria.

The critical assumptions are many: 1) Bacteria are the only important ice nucleating agent at warm (-5°C to 0°C) temperatures; 2) _P. syringae_ is the important INA bacterial species; 3) INA$^-$ and INA$^+$ genotypes are selectively equivalent; 4) Inter-strain competition is greater than intra-strain competition; 5) Leaf epiphytic INA bacteria are the most important source of atmospheric INA bacteria; 6) _P. syringae_ is rare in decaying organic matter; 7) Atmospheric INA bacteria are the major warm temperature ice nuclei; 8) Precipitation is limited by the concentration of warm temperature ice nuclei.

If all these assumptions hold, then the worst effect could be local desertification. High value, frost sensitive crops are frequently grown in large areas surrounding metropolitan areas. A shift in precipitation in these areas could have a major impact on many people. Since many of our assumptions can be easily tested at minimal cost, we believe that they should be tested immediately.

3. Predictions of the potential ecological impact of INA^- bacteria, or of any other biologically engineered microorganism, are based on information about the natural history of these organisms. For microoganisms, and specifically for INA bacteria, this information is severely lacking. In particular, details of competition and predation, dispersal, adaptive value, and ecosystem-level functioning are lacking. We believe that research should be initiated in the basic ecology of microorganisms.

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Chapter 1: Introduction

A worst case analysis of the potential effects from widespread agricultural use of ice-nucleation activity minus bacteria (INA$^-$ bacteria) to protect plants from frost must evaluate the possiblities of spread of the organism. Additionally, this analysis must examine the interactions between INA$^-$ and INA$^+$ bacteria, and evaluate the influence of INA$^+$ bacteria on climatic processes. This analysis is complicated by the multiple spatial and temporal scales under consideration and the relative dearth of knowledge about most of the details of the process.

We will use the following terminology to discuss bacteria with differing abilities to act as ice nuclei. Bacterial populations with some measurable ice nucleation activity have been designated as "INA bacteria" (Lindow et al., 1978a; Ashworth et al., 1985b), and we will refer to them as such in this report. We will use the term "INA$^-$ bacteria" to designate bacterial populations that do not have measurable ice nucleation activity, no matter how the bacteria were produced. When we refer to only genetically engineered INA$^-$ bacteria, this will be specified. Further, a distinction must be made between bacterial populations with ice nucleation activity and individuals with ice nucleating activity. The former will be called "INA bacteria" and the latter described as an "IN bacterium". An individual without ice nucleation ability will be called "IN$^-$ bacterium". These are all phenotypic designations. It will be necessary at times to refer to the genotypic composition of either bacterial populations or individuals. This will be clearly indicated in the discussion and the genotypes will be designated "INA$^+$" or "INA$^-$", disregarding variation in

DNA structure. The abbreviation "INA" will always refer to a phenotypic characteristic of either a population or an individual. While many authors allow "INA" to refer to a process or an action, and use it indiscriminately as a noun, verb, or adjective, we find this use confused.

Ice nucleation ability at -5°C is frequently used to determine if bacteria are INA bacteria. At -10°C many more bacteria strains have ice nucleation activity (Paulin and Luisetti, 1978; Lindow et al., 1978c). Since ice nucleation activity at temperatures approaching zero have been of greater interest than ice nucleation at lower temperatures, we will focus attention on the warmer temperatures.

We first look at a worst case scenerio, where a maximally favorable environment for INA^- bacteria is assumed. From this we estimate the maximum possible load of INA^- bacteria in the atmosphere, and evaluate if an absolute worst case scenerio holds any potentially harmful effects. This worst case scenerio contains the assumptions that INA^- bacteria will be applied to all agricultural land, that they will achieve maximum growth at each stage of the process, and that they will completely displace INA bacteria on crops. While this is a highly artificial scenario, it provides us an opportunity to sketch the structure of our subsequent analysis, and to identify the relative variability possible at each of the steps in the movement of INA^- bacteria from release to transport into the atmosphere.

In the following chapters, each of the steps in the worst case analysis outlined in chapter two will be examined to take into account realistic biological processes. We call this a biologically constrained *worst case analysis*. Information about the biology of bacteria and bacterial analogues will constrain our worst case analysis. As will become evident to the reader, much of this information is still very tentative. Its general validity for INA bacterial species is based on one of three assumptions: laboratory experiments are good field analogues, a particular field experiment is representative of all field environments, and certain other organisms and particles behave similarly to INA bacteria species. The validity of these assumptions is an outstanding ecological problem that has no satisfactory solution. Therefore the biological constraints that we impose upon our analysis should be taken in the spirit of assumptions, not as necessary reality. Our discussion will serve to give greater or lesser credence to these assumptions.

We discuss the types of ice nucleation particles in chapter three. A survey of the available information about the ice nucleation potential in epiphytic bacteria, and the relative importance of the principal INA bacteria, *Pseudomonas syringae* van Hall and *Erwinia herbicola* (Löhnis) Dye, rounds out this discussion.

In chapter four we determine the cropland area that has potential for INA$^-$ bacteria use. We categorize the major crops as frost resistant, chilling sensitive, and frost sensitive, and use United States and world cropland figures for the frost sensitive crops to determine the area of cropland with potential for INA$^-$ application.

We summarize the natural history and life cycles of INA bacteria in chapter five to provide essential background for the subsequent chapters on population dynamics, dispersal, adaptive value, and spread of INA bacteria.

We focus on the natural distribution and overwintering abilities of the primary INA bacteria, P. syringae and E. herbicola.

In chapter six, we consider the population growth of P. syringae and E. herbicola in isolation and in interaction with other components of the epiphytic environment. These components include weather conditions, plant host influences, intraspecific and interspecific competition, and predation. Information on the population dynamics of engineered INA$^-$ bacteria is limited, so we use information on epiphytic bacteria and analogous organisms.

Similarly, our analysis of dispersal in chapter seven must, perforce, rely on data that has been gathered on the dispersal of similar sized particles and organisms. We include information on the dispersal of plant pathogens, pollen, and pollution particles. We examine dispersal on four intergrading spatial scales, starting with movement on leaf surfaces and ending with global movement.

An intriguing and critical question is what is the adaptive value of the INA$^+$ genotype (chapter 8). While it is possible that ice nucleation activity confers no adaptive advantage, two major roles have been suggested for ice nucleation activity in epiphytes: that ice nucleation activity may increase pathogenicity or that it may provide a dispersal advantage. Understanding the adaptive role of ice nucleation activity has important implications for the potential of released INA$^-$ bacteria to displace INA bacteria.

We study the potential for INA$^-$ bacteria to spread and displace INA bacteria in natural ecosystems in chapter 9. No thorough studies have been published on this particular problem, so our analysis relies heavily on theoretical arguments. Three approaches can be identified: the theory of diffusion-reaction systems (Okubo, 1980), epidemiologic theory (Bailey, 1974; Mollison, 1977; Kampmeijer and Zadoks, 1970), and the theory of population genetics (Crow and Kimura, 1970). Estimates of the growth and dispersal of INA$^-$ and INA bacteria parameterize models of the spread of INA$^-$ bacteria in a variety of spatial scales. The essential question examined in this chapter is the potential of INA$^-$ bacterial populations to establish and be present for long time periods after application.

In chapter ten we explore the relationship of ice nucleation to precipitation processes, based on cloud physics and climatic models. Ice nucleation active particles derived from plants (presumably bacteria), may influence the distribution and quantity of rain. In addition, we summarize information on the atmospheric INA bacteria load because the accuracy of the climatic models depends on this value.

In chapter eleven, using potatoes grown in the United States as a model system, we investigate the possible impact of agricultural applications of INA$^-$ bacteria in more realistic agricultural conditions.

Finally, we summarize this report, commenting on the possible affects of widespread use of INA$^-$ bacteria, and the probability that the climate might be affected.

This analysis focuses only on the impact of wide spread release of INA$^-$ bacteria to supress INA bacteria in agricultural crops, and does not consider the impact of other possible technologies, such as bacteriocides. The reason for this restriction is quite simple. All the other technologies simply act to decrease INA populations locally. Only INA$^-$ bacteria have the potential to leave the area of application and interact with INA bacteria in other habitats; only INA$^-$ bacteria have the potential to affect more subtle aspects of INA bacterial population ecology. It is an analysis of these subtler aspects on which our investigation focuses.

Chapter 2: A Worst Case Scenerio

In this chapter we outline a worst case scenerio involving wide spread use of INA^- bacteria. The worst case scenerio is based on one year's world-wide use of INA^- bacteria, with the assumptions of maximum INA^- bacterial growth, complete displacement of INA bacteria on cropland, and one-hundred percent movement up into the atmosphere. The density of these INA^- bacteria in the atmosphere is compared to the probable natural atmospheric concentrations of INA bacteria. This is an extreme example of a worst case scenerio, but provides background for a discussion of the potential risks associated with wide-spread use of INA^- bacteria. It is conceivable that in this extreme worst case, very little environmental impact would be predicted. There might be so many INA bacteria in the atmosphere that altering loading rates from agricultural sources would have little effect. We conclude, however, that agricultural sources can be significant and a more detailed analysis is warranted.

Frost Vulnerable Cropland

To estimate frost vulnerable cropland (see step number 1, in Figure 1), we use data on the total world agricultural cropland area for frost sensitive crops. Extensive establishment of INA^- bacteria on weeds and natural vegetation surrounding cropland from applications of INA^- bacteria is not considered here.

In this worst case scenerio, we use the total world's cropland. The majority of agricultural regions of the world experience frosts, as shown in Table 3 of chapter 3 (Griffiths, 1981; Muller, 1982; Rudloff, 1981; Schwerdtfeger, 1982). Although a large proportion of tropical, and sub-tropical regions are frost free, highland regions in the tropics do experience frosts (Chang, 1968; Rudloff, 1981; Schwerdtfeger, 1982; Takahashi and Arakawa, 1981; Wang, 1972). The total world cropland acreage of frost vulnerable cropland is about 10.6×10^8 ha (Table 3, Chapter 3). The variance over time associated with the estimated cropland is remarkably small (C.V. = 3 percent). The world's cropland area does not change significantly from year to year, although a gradual increase in the total land cultivated is occurring with time (F.A.O. Production Yearbook, 1984). Cropland area on which INA^- bacteria might actually be applied is probably much smaller.

Leaf Area Index

Step two in Figure 1, is to estimate the leaf area associated with the frost vulnerable cropland. This provides the total leaf area on which INA^- bacteria could potentially establish. Leaf area associated with land area is generally expressed as Leaf Area Index (LAI) (Whyte, 1960). LAI can be used as an estimate of the amount of leaf area per land area of crops grown (Crookston et al., 1978; Daughty and Hollinger, 1984) that would be available for INA^- bacteria colonization.

LAI varies from about 0.05 to 12.0, changing with differences in species, crop growth stages, and plant populations (Whyte, 1960). Perennial cropland, such as pastures, tends to have about double the LAI of row crops (Chapman, 1979). Mature stands of small grain crops and row crops have been found to have LAIs that range from approximately 2 to 5. We use an LAI of 5, since the maximum LAI for crops, excluding pasture situations, is about 5 (Stotskopt, 1981; Wiegand and Richardson, 1984). Therefore, the total leaf area theoretically available to INA^- bacteria is:

$$10.6 \times 10^8 \text{ ha} \times 5 \text{ LAI} = 53.0 \times 10^8 \text{ ha.}$$

Similar to the variability associated with cropland area in step 1, LAI does not vary much. About five-fold variation occurs in maximum LAI for mature rowcrops and pastures (Stotskopt, 1981). Very low LAI's, which are associated with seedlings, are highly transient.

INA$^-$ Bacteria Populations on Leaves

The population density of INA$^-$ bacteria per leaf (step 3, Figure 1) can vary greatly with environmental conditions, and densities will apparently vary with measurement techniques. A representive example of the range of populations present is provided by surveys of INA$^-$ bacteria populations on leaf surfaces, which have found maximum leaf populations ranging from 100 cells/gfw (gram fresh weight) on citrus leaves to over 10^7 cells/gfw on almond and walnut leaves (Lindow, 1982b; Lindow, 1983a). To translate from grams fresh weight to leaf area, we calculate approximately 100 cells/45 cm^2 on citrus leaves to over 10^7 cells/50 cm^2 on almond and walnut leaves (based on data in Leben and Whitmoyer, 1979).

For this worst case estimate, we used the maximum population figures per leaf area. The highest reported population for an INA bacteria species on plant surfaces is that of P. syringae on bean leaves, 10^8 cfu / 50 cm^2 leaf area (cfu = colony forming units) (Hirano and Upper, 1985b; see Appendix 1). This is equivalent to 2×10^{14} cfu / ha surface area and:

$$2 \times 10^{14} \text{ cfu/ha} \times 5.3 \times 109 \text{ ha} = 10.6 \times 10^{23} \text{ cfu on all crops.}$$

We assume that the INA$^-$ bacteria applied to cropland will be effective at preventing frost and therefore effective at supressing naturally occurring INA bacteria. Thus, in this scenario, atmospheric IN bacteria would come primarily from natural vegetation.

The population size of INA bacteria species per leaf has the potential to vary by over six orders of magnitude (Appendix 1). This is the largest source of variance in our worst case analysis. Knowledge about epiphytic bacteria growth and spread rates is critical to assess accurately the possible influence of INA bacteria on climate.

Bacteria Loading Rate into the Atmosphere

Little data is available on the movement of bacteria from leaf surfaces into the atmosphere, step 5 in Figure 1 (Akers et al., 1979; Bovallius et al., 1980). We assume here that all of the epiphytic INA$^-$ bacteria on cropland will move into the atmosphere. More detailed information on the loading of bacteria into the atmosphere would allow a more reasonable, smaller estimate of the overall proportion of epiphytic bacteria that move into the atmosphere and survive. However, if the epiphytic bacterial population is very large compared to the total number of bacteria in the atmosphere, then even a small proportion moving into the atmosphere could have a significant effect.

Comparison and Consequences

We estimate the atmospheric concentration of naturally occuring INA bacteria species in the troposhere to compare with INA$^-$ bacteria loadings. We use the troposphere volume rather than the total atmosphere volume, since the troposphere is by definition where weather occurs, and the weather is our ultimate concern. We calculate the troposphere volume to be about 7.7×10^{18} m^3 (Appendix 1).

Unfortunately the data on atmospheric concentrations of naturally occurring INA bacteria are extremely limited. For this scenario, we will use two values of bacteria atmospheric concentrations: 40 INA bacteria per m^3 and 1000 bacteria per m^3 of atmosphere (Flanagen and Jayaweera 1980; Schnell et al., 1981). Therefore, we estimate that there are from 3.08×10^{20} to 7.7×10^{21} INA bacteria in the troposphere.

Comparing these numbers with our calculated, worst case estimate of INA^- bacteria production on cropland, we note that the 10.6×10^{23} cfu bacteria produced is three to four orders of magnitudes larger than the total bacterial load in the troposphere, 3×10^{20} or 8×10^{21} INA bacteria.

Our final assumptions in this analysis are about recolonization and interaction of bacteria on leaf surfaces. The first is that the composition of INA^- and INA bacteria on natural plants next year will be roughly proportional to the composition of bacteria in the atmosphere. In other words, the composition of atmospheric populations influences the composition of epiphytic populations to some degree. The second is that IN^- and IN bacteria are competitively equivalent. Thus both production in natural environments and bacterial loading rates into the atmosphere will be affected by agricultural sources of INA^- bacteria.

These assumptions give rise to a model that predicts a gradual decline in INA bacteria in the atmosphere (see Appendix 2). In addition, the greater the agricultural load the faster the disappearence of INA bacteria in the atmosphere. Our worst-case estimate of INA^- bacterial loading from agricultural sources suggests that replacement of INA bacteria in the atmosphere might be fast. So if INA bacteria play an important role in precipitation patterns, it is conceivable that wide-scale agricultural release of INA^- bacteria could affect precipitation.

Summary

This worst case scenerio illustrates a possibility for climatic impact of wide-spread application of modified INA^- bacteria. The potential pool of INA^- bacteria available to move into the atmosphere could be three orders of magnitude greater than the assumed atmospheric INA bacteria load and could replace natural INA populations. This result may seem to be cause for concern, but we believe that circumstances will not fulfill the assumptions of this worst case scenario. Instead, we conclude that the possibility of climatic impact cannot be dismissed as out of hand, and that a biologically constrained worst case scenario should be developed. The remainder of this report is dedicated to this program.

The scenerio developed in this chapter identifies the variability associated with each process. The amount of world cropland area was found to remain relatively stable over time. In contrast, the range of population densities of INA bacteria associated with cropland area varied by many orders of magnitude, and could be a significant source of error. Further, information on how INA^- bacteria replace INA bacteria on leaf surfaces is lacking. This has important implications for modeling the impact of INA^- bacteria.

There is a pressing need for better information about the quantity of INA bacteria in the atmosphere. Data on the total atmospheric load, and on local air concentrations of INA bacteria are extremely limited (Schnell, 1981). This dearth of representative atmospheric concentration data severely limits our attempt to evaluate the environmental effect of epiphytic INA^- and INA bacteria.

Chapter 3: Sources of Ice Nucleation Activity

Many particles nucleate ice formation. Prior to discussion of INA bacteria, we compare the ability of various particles to nucleate ice formation. Methods to detect ice nucleating activity are many and varied, so we start with a discussion of methods. Then we review the types of particles that nucleate ice and the temperatures at which they nucleate. Finally, we identify the bacterial species capable of nucleating ice at warm temperatures and describe the habitats they come from.

Methods

There are a variety of techniques available for evaluating ice nucleating activity (Vali,1971; Makino, 1982; see Appendix 2). In all of these methods, the material to be tested is cooled, and the temperature at which ice appears is recorded. Some effort has been made to standardize these techniques, but it is difficult and sometimes misleading to generalize from one to another.

Even for a single method, many factors affect its accuracy. First, bacteria do not always nucleate ice at a fixed temperature and time (Maki et al., 1974). Individuals might differ in their ability to nucleate ice or a given individual might only occasionally express an ice nucleation phenotype. Second, environmental and cultural conditions *in vitro* affect the expression of ice nucleating activity. Incubation temperature for *P. fluorescens* cultures (Maki and Willoughby, 1978), carbon source supplied to *Erwinia herbicola* (Lindow et al., 1978b), growth medium composition, and age of culture (Lindow et al., 1982b) affect ice nucleating activity *in vitro*. Third, non-viable bacteria retain ice nucleation activity (Maki et al., 1974). Further, while cloud chamber techniques duplicate the actual ice nucleation situation in clouds most closely, repeatability is much lower than that of the droplet freezing technique, and the error is higher (Maki and Willoughby, 1978). Cloud chamber techniques are useful for confirming that ice nuclei are active in the atmosphere.

The length of time of incubation and the size of the sample being incubated can affect the measurement of ice nucleating activity (Ashworth et al., 1985a; 1985b). If few ice nucleating particles are in a sample, then nucleation events may be stochastic and accumulate over time. If, however, many ice nucleating particles are in a sample, then nucleation events will be deterministic and accumulate almost instantaneously. Hirano et al. (1985) observed little time dependence in ice nucleation events; 95 percent of freezing occurred in the first half hour of a 4 hour test. Ashworth et al. (1985a; 1985b) observed nucleation events to accumulate over time. While Hirano et al. (1985) suggested that time accumulated nucleation might result from mishandling samples, differences in either the source or density ice nucleating particles may account for the differences in results of the two groups. Ashworth et al. (1985a; 1985b) had samples with high "intrinsic" ice nucleating activity and very low INA bacteria populations (less than 100/gfw), while Hirano et al. (1985) had samples with high INA bacterial populations. This issue remains to be resolved.

It is difficult and sometimes misleading to compare results of different authors. Despite this, it is necessary to compare these results to evaluate effectively the potential impact of INA$^-$ bacteria. In the remainder of this report we will be comparing the results of several laboratories. However, since their methods have not been adequately compared, it is impossible to analyse for methodologically induced errors in our interpretations of the data. This restriction on our analysis should be continually borne in mind.

Source-Temperature Relations

All particles that nucleate ice have a characteristic temperature spectrum. Above certain temperatures they cannot nucleate ice. We summarize this data on the temperature relations of inorganic and organic sources of ice nucleation activity in the atmosphere and on plant surfaces (Table 1).

Clay particles had been implicated as important atmospheric ice nuclei; this was supported by the discovery of soil particles at the centers of naturally occurring ice crystals (Kumai, 1961). However, ice crystals were observed in clouds when temperatures were warmer than -5°C, while -9°C was the warm temperature limit at which inorganic soil particles can nucleate (Table 1) (Pruppacher and Kleff, 1982; Rogers, 1979; Schnell and Vali, 1976). This implied that other, warm temperature ice nuclei existed.

Schnell and Vali (1976) found biological ice nucleating agents that acted at warmer temperatures (Table 1). Organically derived ice nucleators associated with decaying leaf litter and oceanic phytoplankton were found to form ice in cloud chamber tests at temperatures as warm as -1°C (Schnell and Vali, 1976). The various organically derived ice nuclei were living and dead bacterial cells, (Table 2; Vali et al., 1976; Maki and Willoughby, 1978; Fall and Schnell, 1985). Bacteria are now considered to be the main source of warm temperature ice nuclei (Lindow 1983b).

Table 1. Ice nucleation activity sources

Source	Temperature	Method	Reference
Inorganic			
Kaolin (Clay)	-15° to -9°C	1,3	12
Kaolin + Organic Unknown	-6° to -4°C	1,3	12
Meteorite Dust Aerosols	-40° to -15°C	2	10
Inorganic Soil Particles	-40° to -15°C	1	10
Silver Iodide	-4°C	1,11	10,15
Flurophlogopite (Synthetic Mica)	-3° to -0.7°C	3,11	9,15
Organic			
Cells, No Extracellular Ice			
Human Red Blood Cells	-40°C	7	3
Yeast Cells	-30°C	7	3
Plant Cells (Soybean)	-30°C	7	3
Amino Acids			
Aspartate	-6°C	10	7
Tryptophan	-5.5°C	10	7
Leucine	-4.5°C	10	7
Kaolin + Aspartate	-6.5°C	10	7
Phenazine	-3°C	11	15
Metaldehyde	-7° to -3°C	3	9
Acetoacetanilide	-7° to -1°C	3	9
Soil Suspension (Potatoes Grown in)	-9° to -4°C	3	9
Leaf Derived Nuclei = Org.			
Unknown from Leaf Litter	-20° to -4°C	1,3	12,14
Aerosols from Leaf Litter	-15° to -6°C	4	13
Associated: Marine Plankton	-15° to -4°C	1,3	11
Bacteria	-40° to -0.5°C	1-11	Table 2
Unknown			
Intrinsic (No Bacteria)			
Stem + Fruit	-2.5°C	9	8
Stem	-4° to -2°C	6,8,9	1,2,5
Fruit	-7°C	9	8
Stem Extract	-5.5°C	9	2
Intrinsic (with INA Bacteria)			
Stem + Fruit	-3°C	9	8
Stem	-3°C	9	5
Fruit	-3°C	9	8
Intrinsic (Bacteria?)			
Leaf	-6° to -2°C	9	6
Stem	-15° to -5°C	9	6

References

1. Ashworth and Davis, 1984
2. Ashworth, et al., 1985b
3. Franks, et al., 1983
4. Gokhale and Gould, 1969
5. Gross, et al., 1984
6. Kaku, 1973
7. Power and Power, 1962
8. Proebsting, et al., 1982
9. Rajashekar, et al., 1983
10. Rogers, 1979
11. Schnell, 1975
12. Schnell, 1977
13. Schnell, and Tan-Schnell, 1982
14. Schnell and Vali, 1976
15. Yelenosky, 1983

Methods

1. Cloud Chamber (Schnell and Vali, 1976)
2. Cloud Chamber and Filter (Gokhale and Gould, 1969)
3. Droplet Freezing (Vali, 1971; 1977)
4. Modified Droplet Freezing (Schnell, 1979)
5. Leaf Discs (Lindow, 1982b)
6. Test Tube Freezing (Yankofsky, et al., 1981)
7. Differential Temperature Analysis (D.T.A.) of Cell Freezing (Franks, et al., 1983)
8. Temperature Differences in Trees, Measured by D.T.A. (Ashworth and Davis, 1984)
9. Temperature Differences in Shoot Pieces, measured by D.T.A. (Gross, et al., 1984)
10. Aerosol Freezing in Cold Box (Power and Power, 1962)
11. Percent of Attached Tree Leaves Freezing (Yelenosky, 1983)

Table 2. Highlights of the identification of bacterial ice nucleation.

Bacterium	Source	Number of Isolates	Reference
P. syringae	decaying leaves	1	Maki et al., 1974
P. fluorescens	primarily water		Maki and Wiloughby, 1978
P. syringae	primarily water	37 total	
Gram positive coccus	from water		
P. syringae	epiphytes on	>760	Lindow et al., 1978a
E. herbicola	various plants	>240	
E. herbicola	corn leaves	1	Lindow et al., 1978b
P. viridiflava	culture collection	8	Paulin and Luisetti, 1978
P. syringae	*Prunus cerasus*	51	Latorre et al., 1979
P. mors-prunorum	*Prunus cerasus*	23	
P. syringae	crop plants	123	Lindow, 1982b
E. herbicola	crop plants	43	
P. syringae	Fruit trees	82	Gross et al., 1983
P. fluorescens	marine	1	Fall and Schnell, 1985
unknown (probably *P. flourescens*	polar sea ice	1	Parker et al., 1984
X. campestris pv. *translucens*	data not avail.		L. Kim et al., in press

Warm Temperature INA Bacteria Species

Some of the INA bacteria found in a wide range of environments have been identified and studied. The species found to date are *Pseudomonas syringae* (Fresh, 1973), *Erwinia herbicola* (Lindow et al., 1978b), *Pseudomonas fluorescens* (Maki and Willoughby, 1978), *Pseudomonas viridiflava* (Paulin and Luisetti, 1978), *Erwinia stewartii* (Wallin et al., 1979), and *Xanthomonas campestris* pathovar *translucens* (L. Kim et al., in Lindow, 1985). All of these species also have strains that do not nucleate ice. Ice nucleating activity is most frequently found in *Pseudomonas syringae*. *Erwinia herbicola* also has ice nucleating activity, but at nearly 10 percent the intensity of *P. syringae* (Lindow 1983b).

It is not known if all the major bacterial species active as ice nuclei have been identified. Only *P. syringae* and *E. herbicola* have been repeatedly and commonly found. Because there are a vast number of untested strains and species of bacterial epiphytes on plants (see Appendix 4), there may be more INA species.

The frequency that INA bacteria strains have been discovered in nature can indicate the likelihood of a new INA species being discovered. If we assume that the strains tested represent a random sample of all testable strains in nature, we can construct odds ratios, and estimate the odds of discovering more INA species. While it is clear that the INA composition of bacteria populations changes with time (see Chapter 6), this provides a first approximation.

Paulin and Luisetti (1978) tested 387 bacterial isolates in 6 genera. They found that 119 (31%) of the isolates were active ice nuclei at

$-4^{\circ}C$. All INA isolates were Pseudomonas spp. except for one Erwinia ananas (= Erwinia herbicola pv. ananas) isolate. None of the 51 non-fluorescent Pseudomonas isolates were ice nuclei. None of 52 Erwinia herbicola isolates were ice nuclei. No Xanthomonas campestris or Pseudomonas fluorescens isolates were tested.

Lindow (1978a) tested isolates of epiphytic bacteria from 95 plant species in several states. Of these, 74 species had epiphytic INA bacteria on them. All INA isolates were found to be Pseudomonas syringae or Erwinia herbicola. INA bacteria were <0.01 (Natal Plum in Florida in winter) to 95 percent (Wisconsin, Acer negundo, young leaves in May) of the total bacterial epiphytic population. Lindow (1982b) later tested 256 bacterial isolates. Of these 141 were pseudomonads, mostly P. syringae, 55 were Erwinia spp, mostly E. herbicola, and the rest were assorted plant pathogens, epiphytes, and miscellaneous bacteria. All INA isolates were P. syringae or E. herbicola, except for one P. fluorescens.

Quantitative data on the incidence of INA isolates in natural populations are sparse (Appendix 4). For example, the frequency of INA strains in E. herbicola and P. fluorescens is uncertain. These data show that P. syringae has a high percentage of detectable ice nucleation activity in natural populations (Table 3), while E. herbicola has a low frequency. The odds of finding a new species of INA bacteria are between 1/500 and 1/50 the probability of finding another strain of INA P. syringae (Table 4), given that a similar number of isolates are tested. Since most species are rare compared to P. syringae, P. fluorescens, and E. herbicola, they may be hard to detect. Their existence, however, cannot be ruled out.

In summary, by its ubiquity and high frequency of ice nucleation, _P._ _syringae_ is probably the largest source of bacterial ice nuclei. However, both _E._ _herbicola_ and _P._ _fluorescens_ are also important sources of ice nuclei. Other species are likely to have INA strains, but these may be relatively less common. The importance of these sparse species should not be diminished by their sparseness; they may be very effective ice nuclei on plants.

Table 3. Frequency of recovery of INA isolates in nature, summarized from Appendix 4[1].

Species	Number of Tested Isolates	Number of INA Isolates	Percent INA
Pseudomonas syringae[2]	648	353	54.5
Erwinia herbicola[3]	62	3	4.8
Pseudomonas fluorescens[4]	4	1	25.0
Pseudomonas viridiflava[5]	19	8	42.1
All Others[6]	256	2	0.8

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[1] When ice nucleating activity was reported by more than one research group, the species is listed separately.

[2] Does not include data from Lindow et al., 1975, *P. syringae*-like isolates reported in Lindow et al., 1982, and data from Gross et al., 1984.

[3] Does not include *E. herbicola*-like isolates reported in Lindow et al., 1982.

[4] Evidence for ice nucleation much stronger than apparent here.

[5] Anderson and Ashworth (in press) confirmed Paulin and Luisetti's (1978) report.

[6] *E. stewartii* and HK'-31 positive for ice nucleation.

Table 4. The odds of finding an INA isolate in a new species compared to the odds of finding an INA strain in an already identified species, given that a similar number of isolates are tested.

	Odds of Finding New Species
Compared to	Based on Table 3
Pseudomonas syringae	0.014
Erwinia herbicola[1]	0.16
Pseudomonas fluorescens	0.03
Pseudomonas viridiflava	0.02

[1] The odds of finding INA *E. herbicola* are calculated to be 0.088 that of finding INA *P. syringae* given that a similar number of isolates are tested. Lindow et al., (1978) claimed that INA *E. herbicola* occured at 85 cfu/gfw, while INA *P. syringae* occured at 1000/gfw, when both species were equally abundant. Thus the odds of finding INA *E. herbicola* are 0.007 that of finding INA *P. syringae*.

Other Warm Temperature Ice Nuclei

The importance of bacteria to ice nucleation on plant surfaces has been debated by researchers who have found non-living sources of ice nucleation on plant tissue. The presence of "intrinsic" ice nuclei in plant tissue has been suggested by numerous authors (Anderson et al., 1982; Ashworth and Davis, 1984; Ashworth et al., 1985b; Gross et al., 1984; Kaku, 1973). Their existence is indicated by autoclave experiments, by the lack of detectable INA bacteria populations, by ice nucleation activity after extensive washings of plant tissue or exposure to bacterial inhibitors (Anderson et al., 1982; Ashworth et al., 1985b), and by comparison of intact and homogenized plant tissue (Kaku, 1973). Only the last method is conclusive. Ashworth et al., (1985a) suggested that non-living warm temperature ice nuclei exist in plant tissues. However, no mechanisms have been suggested yet (Ashworth, personal communication).

Crystals of several organic compounds can be ice nuclei at temperatures warmer than -5°C, including steroids (Fukuta and Mason, 1963; Head, 1986), amino acids (Barthakur and Maybank, 1963; Parungo and Lodge, 1967; Power and Power, 1962), proteins (Zettlemoyer et al., 1961), terpenes (Rosinski and Parungo, 1966), metaldehyde (Fukuta, 1963), and -phenazine (Head, 1962). These compounds are active only in crystaline form (Parungo and Lodge, 1965), and lose activity when dissolved. Crystals of these compounds are unlikely to occur in nature. Fluorophlogopite, a synthetic mica, nucleated ice at temperatures as warm as -1°C (Rajashekar, et al. 1983). However, this substance does not occur in nature.

Complicating our understanding of possible non-living ice nuclei, is that bacterial ice nucleating activity is retained by nonviable bacteria

(Lindow and Connell, 1984; Maki et al. 1974; Vali et al. 1976). For example, antibiotics lethal to P. syringae, but not disruptive to bacteria cell walls, did not decrease ice nucleating activity (Vali et al. 1976). Similarly, freezing ability was associated with the cell membrane fraction of disrupted INA pseudomonads (Maki and Willoughby, 1978), and bactericide treatments of leaf surfaces and in culture showed that P. syringae did not lose immediately its ice nucleating activity (Lindow and Connell, 1984). Lindow (1983a) suggested that ice nucleation by dead bacteria may be a widespread phenomenon facilitating plant freezing.

A bacterially produced, membrane associated protein probably initiates ice nucleation (Maki and Willoughby, 1978; Kozloff et al., 1984). The importance of proteins is suggested by the type of inhibitors that inactivate ice nucleation without killing the bacteria. These inhibitors include pH extremes, copper and zinc solutions, smoke, and some cationic detergents (Lindow, 1983b; Lindow and Connell, 1984). A carbohydrate, phosphatidylinositol, may be a part of the membrane ice nucleation sites on INA bacteria, possibly associated with a protein (Kozloff et al., 1984). Since a membrane protein can in some cases retain its integrity after the death of the bacterium, this allows nonviable bacteria to retain ice nucleation ability. Further evidence that ice nucleation in P. syringae is a protein/membrane complex, was gathered by cloning and expressing P. syringae INA^+ DNA in E. coli (Orser et al., 1983; 1984).

Existence of large quantities of non-viable warm temperature ice nucleating sources would reduce the effectiveness of agricultural use of INA- bacteria and could reduce the impact of loss of INA bacteria in the atmosphere. Given the uncertainty surrounding these sources, in our analysis, we will assume that they are insignificant.

Habitats of INA Bacteria

INA bacteria are found in both marine and terrestrial habitats. Marine bacteria are associated with upwellings (Schnell and Vali, 1976), where algal growth is rapid. Ice nucleating activity may be as high as $10^7 - 10^8$/gram of plankton ($-10^\circ C$, Schnell and Vali, 1976). One species of algae had ice nucleating activity, *Heterocapsa* (= *Cachonina*) *niei* (Schnell, 1975). This alga was associated with an INA bacterium, which was phenotypically similar to *P. fluorescens* biotype G (Fall and Schnell, 1985). Parker et al. (m.s.) also tested several marine algae and bacteria, and found that only one bacterial strain formed ice nuclei at warm temperatures. As far as can be determined, all marine sources of warm temperature ice nucleation are bacterial.

Terrestrial sources of INA bacteria are many. The two most important are probably leaf tissue and decomposing organic matter. Schnell and Vali (1976) report that decomposing vegetation was the more potent source of INA bacteria. They found no detectable amounts of ice nucleation (at $-5^\circ C$) on living poplar leaves and $10^3 - 10^6$/gdw on decomposing vegetation. Further, they showed that decomposition lead to increases in ice nuclei. These results clearly implicate living organisms, but poplar may have been a poor comparison. Lindow et al. (1978a) reported high levels of INA bacteria on a number of plant species. These densities varied from not detectable to $10^{7.4}$/gfw. Poplar had low population densities of only 0 to $10^{3.2}$/gfw. These patterns will be discussed furthur in chapter 5.

All three habitats, marine, vegetation, and decaying organic matter are potent sources of INA bacteria. These sources all probably affect concentrations of atmospheric warm temperature ice nuclei, at least locally. Thus the impact of wide-spread release of INA⁻ bacteria for agriculture must either take all these sources into account or develop a local analysis.

Summary

All particles can nucleate ice formation if it is cold enough. At temperatures warmer than -5°C, however, there are very few sources of ice nuclei. The main types are living bacteria, dead bacteria, some inorganic particles, some crystaline chemicals, and "intrinsic" plant structures. Living INA bacteria are certainly important sources, and crystaline chemicals are certainly not. Inorganic particles are believed to be unimportant, but this has not received as much attention. The role of dead bacteria and intrinsic plant structures is still disputed.

The most important INA bacteria species are probably _Pseudomonas syringae_, _P. fluorescens_, _Erwinia herbicola_ and _P. viridiflava_. There are, however, probably many more INA species. These four are important because they are extremely common and frequently active as ice nucleators. _P. syringae_ is particularly notable because of its high abundance and high frequency in which INA isolates occur. Other species should not be dismissed for their sparseness. There may be some potent INA species that play important roles in plant freezing.

INA bacteria are found in both marine and terrestrial habitats. Global analysis of the impact of widespread releases of INA⁻ bacteria must consider both sources. Local analysis could restrict attention to terrestrial sources, both living and decaying vegetation.

To continue a worst case scenario, we will assume that INA bacteria are the major source of ice nuclei above -5°C. We must, however, consider the roles of P. fluorescens and E. herbicola and the importance of marine and humic sources.

Chapter 4: Frost Vulnerable Crops and Cropland

We estimate the area of the frost vulnerable cropland of the United States and the world to determine the total cropland that could conceivably have INA$^-$ bacteria applied to it. We determine this area in two steps. First, we classify crops as frost sensitive or not. Frost sensitive crops might increase yield by frost avoidance. Second, we estimate the area associated with these crops.

There are several other ways to determine the area of cropland on which INA$^-$ bacteria could be applied. One could analyze losses claimed from frost damage to determine the appropriate crops and regions that are frost vulnerable. One could analyse, crop by crop and region by region, the potential for frost damage; this, however, is beyond our means. One could also analyse the potential economic returns from using INA$^-$ bacteria to control frost. All of these methods may seem more realistic than the method we chose, but our method is in the spirit of worst case analysis.

Vulnerable Crops

We determine which crops have the potential for increased yields from use of INA$^-$ bacteria by categorizing each crop as frost sensitive, chilling sensitive, or frost resistant. These categories may describe a continuum of damage to plants by cold temperature stress. If, as has been strongly suggested in recent review articles (Levitt, 1980; Lyons, 1973; Steponkus, 1984), damage to plant membranes is largely responsible for the injury found in both frost and cold temperature damage, the difficulty in separating these phenomena becomes understandable.

Chilling injury is crop injury caused by exposure to non-freezing temperatures; the critical cold temperature that generally causes damage has been identified as 10^{o} to 12^{o}C (Lyons, 1973); or broadly defined as 0^{o} to 15^{o}C (Levitt, 1980). Recently, Lindstrom and Carter (1985) pointed out that chilling injury could be defined as injury caused by cold temperatures in the absence of freezing damage; this would include injury by temperatures colder that 0^{o}C, where ice formation had been prevented. While information about chilling injury under subzero conditions would be extremely useful for estimating the potential for effective use of INA^{-} bacteria, unfortunately few such studies are available (Lindstrom and Carter, 1985).

Frost sensitive crops include those injured by temperatures in the range of -1^{o} to -3^{o}C; concomitantly frost resistant crops are those not experiencing injury at -1^{o} to -3^{o}C (Burke et al., 1976). Plant injury from temperatures approaching zero is often considered synonymous with damage from a "killing frost" (Burke et al., 1976), and thus, "frost" sensitivity and resistance in crops is usually determined by observing the effect of subzero temperatures on plants, rather than attempting to separate out frost and cold injury effects. This lack of data on frost sensitivity and frost resistance restricts our analysis. Instead, we categorize crops mainly using information about crop temperature sensitivity.

We expand the category of frost sensitive crops to include any crops that are limited in production or geographical range by frosts whether or not they experience any chilling injury (Table 5). For example, we include bananas in Table 5. Banana yields are severely reduced by temperatures of 12^{o}C (Lyons, 1973), and are very sensitive to chilling. The yield of banana plantations, however, is at times limited by frost, apart from cold temperature effects (Williams, 1975). Since the possibility exists that bananas could be candidates for applications of INA^- bacteria, the total banana acreage is included in Table 5. Crop plants that are restricted in geographical range and limited in yield only by cold temperatures are not included as frost sensitive crops (see Table 6). An example is rice. However, crops that are frost susceptible even during a brief period of growth and development were included as frost sensitive.

Table 5 Frost vulnerable cropland.

Frost Vulnerable Crop	References[1]	US Acreage[2] 1000 HA	World Acreage[3] 1000 HA
Cereal, Pulse, and Root Crops			
Wheat, Spring[5]	3,5,20	31,540	228,094
Barley[5]	3,20	4,218	78,581
Oat[5]	20	5,648	25,526
Corn	3,5,7,16	29,555	129,627
Millet	5	81	42,351
Sorghum	5,19	6,486	49,004
Pulses	1,3,5,12,15	29,339	67,837
Potato[5]	3,5,13,15	513	20,303
Total		107,380	645,099
Vegetable Crops			
Artichoke	3	5	120
Carrot	11,15	34	542
Corn, Sweet	3,5,16	260	N.A.[4]
Beans, Green	5,12,15	83	422
Melons	10,18	10	2,451
Peppers	10,18	37	989
Cucurbits (Squash, etc.)	3,15,18	21	1,365
Tomato	1,2,5,10,14	168	2,524
Total		618	8,413
Specialty Crops			
Coffee[6]	19	1	10,062
Tea[6]	19	N.A.	2,788
Sugar Cane[6]	12,19	304	15,895
Tobacco	5	369	4,155
Grapes (Raisins, Wine)	3	354	11,795
Total		1,028	44,695
Tree Fruit and Nuts			
Avacado	10	35	105
Banana (Including Plantain)[6]	10,19	0	4,092
Berries: Strawberries		20	70
All other Berries	3	31	N.A.
Citrus Fruit (Orange, Lemon, Grapefruit, etc.)	8,10,21	521	3,740

Table 5 (continued)

Frost Vulnerable Crop	References[1]	US Acreage[2] 1000 HA	World Acreage[3] 1000 HA
Papaya	19	1	140
Stone Fruit and Nuts (Peach, Apricot, Almond)	4,6,9,10	301	633
Pome Fruit (Apple, Pear, Plum, Cherry)	4,10,20	406	3,704
Pineapple[6]	10,19	15	583
Total		1,330	13,067
Total Vulnerable Crop Area:		110,356	711,274

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[1] References listed by number on following page.

[2] U.S.D.A. 1982 Agricultural Census Data; U.S.D.A. 1982-1984 agricultural statistics.

[3] F.A.O. 1985 Production Yearbook: 1984 data, for total world production.

[4] N.A. = Data not available

[5] Crops that are frost resistant in some growth stages.

[6] Crops that experience chilling injury.

References

1. Anderson et al., 1982
2. Anderson and Ashworth, 1985
3. Burke et al., 1976
4. Gross et al., 1984
5. Hellmers and Warrington, 1982
6. Klement et al., 1984
7. Lindow et al., 1982
8. Lucas, 1954
9. Lindow and Connell, 1984
10. Lyons, 1973
11. Modlibowski and Pisek, 1973
12. Matheson, 1979
13. Rajasheker et al., 1983
14. Sellschop and Salmon, 1928
15. Spector, 1965
16. TeVelde, 1984
17. Thomson, 1979
18. Tindall, 1983
19. Williams, 1975
20. Wilsie, 1962
21. Yelenosky, 1983

Table 6. Frost resistant and chilling sensitive crops (Not candidates for use with INA$^-$ bacteria).

Crop[1]	Frost Resistant	Chilling Sensitive	Reference
Rice	-	+	1,4,8
Cotton	-	+	1,4,6
Rye	+	-	1,5
Cassava	-	+	5,7
Cabbage Family	+	-	1,2,5
Sunflower	+	-	1,6
Sugar Beets	+	-	1
Sweet Potato	-	+	5,7
Sesame	-	+	3
Cocoa	-	+	2,8
Yams	-	+	7
Jute	-	+	7
Onions, Garlic	+	-	5,7
Safflower	+	-	1,6
Hemp	+	-	1
Eggplant	-	+	5,7
Rubber	-	+	8

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[1] Crops are arranged by the quantity of the world's cropland that is associated with each crop, the largest being listed first.

References

1. Hellmers and Warrington, 1982
2. Modlibowski and Pisek, 1973
3. Matheson, 1979
4. Sellschop and Salmon, 1928
5. Spector, 1965
6. Thomson, 1979
7. Tindall, 1983
8. Williams, 1975

Frost resistance and cold temperature resistance varies for every plant, changing with different growth stages and increasing with cold acclimation. Variation in frost resistance is seen with different cultivars (Burke et al., 1976; Lyons, 1973). Keeping with a worst case scenerio, crops in which at least some varieties are frost sensitive are categorized in their entirety as frost vulnerable crops and were included in Table 5. For example, all potato cultivars are not frost sensitive (Lindstrom and Carter, 1985), yet Table 5 lists total potato acreage.

Vulnerable Cropland Area

We estimate the cropland area associated with each of the crops in Table 5. Essentially 100% of the cropland in the United States experiences frost, so the total U.S. cropland of each frost vulnerable crop was used. Clearly, this overestimates the actual crop area that INA^- bacteria might be applied. In northern latitudes, frost comes so near the end of the growing season that little yield loss can be anticipated. In addition, many crops experience early frosts only in restricted habitats. For example, valleys are more likely to experience frosts than hills (Wang, 1972).

The total world crop acreage, however, does not experience frost. Approximately 27% of the world's cropland is frost-free (Table 7).

Table 7. Frost-vulnerable cropland of the world.

Region	Total Cropland 1000 HA	Frost-Vulnerable Cropland 1000 HA	(Percent of Total)
Africa	183,059[1]	52,861[1]	(28.9%)[2]
North and Central America	272,989	247,995	(90.8%)
South America	138,694	74,944	(54.0%)
Asia	456,037	267,097	(58.6%)
Europe	140,366	140,366	(100.0%)
Oceania	48,196	48,196	(100.0%)
U.S.S.R.	232,390	232,290	(100.0%)
World	1,471,731	1,063,849	(72.3%)
World 4 Year Average	1,457,406 ±1%	1,058,515±3%	(72.7%±1%)

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[1] Based on FAO estimates of 1984 arable and permanent cropland.

[2] Percentage of regional frost-vulnerable cropland per total cropland. Frost-vulnerable zones determined after Muller, 1982; Rudloff, 1981; and World Survey of Climatology, Volumes 5 - 13.

Specifically, tropical and subtropical regions are relatively frost free and so might be considered of little interest for potential use of INA- bacteria. However, tropical highlands experience frosts. In general, temperature decreases by about 1.8°C for every 300 m rise in altitude (Webster and Wilson, 1980). Further, the infrequent frosts that occur in tropical highland regions and in subtropical regions can be catastrophic for perennial agriculture. Although frost might occur but once in ten years, production may be devastated in frost sensitive varieties of crops such as coffee, tea, banana, and sugarcane (Chang, 1968; Schwerdtfeger, 1982; Webster and Wilson, 1980). Recent increases in coffee prices, from frost damage in coffee growing regions illustrate the dramatic impact frost can have on a tropical and subtropical crop. In fact, infrequent frosts are mentioned as a significant agricultural problem in large tropical and subtropical regions of Central and South America, Africa and Asia (Chang, 1968; Griffiths, 1981; Muller, 1982; Schwerdtfeger, 1982; Takahashi and Arakawa, 1981).

The majority of the total world crop acreage does experience frosts at least occasionally. In tropical and subtropical regions frosts can still affect agriculture. Thus, the world cropland area data for each crop, as reported in the F.A.O. Production Yearbook, was used as a rough, but not unreasonable estimate of the frost vulnerable cropland area.

A small number of crops contribute substantially to our estimate of the total area of frost vulnerable cropland. Wheat is the cereal grown over the largest area, and accounts for about one-third of the total acreage of frost vulnerable crops (Table 5). Wheat is largely frost resistant to temperatures as cold as -7°C (Hellmers and Warrington, 1982).

However, frost injury may occur at flowering and during grain fill, at approximately -2°C (Hellmers and Warrington, 1982). INA$^-$ bacteria might conceivably be used to avoid frost induced shriveling of kernals. Although wheat is grown world wide, wheat raised in parts of Canada, U.S.S.R and Australia might have the greatest potential for frost problems. These regions contribute about one-third of the world's production of wheat. Considering the difficulty of determining, on a world wide basis, which wheat producing areas might be vulnerable to frosts, we included the total wheat cropland (Table 5).

Corn is the next biggest single contributor to frost vulnerable crop acreage, for both the United States and the world (Table 5). For temperate regions, frost injury in corn can be a problem at both the begining and at the end of the growing season (Hellmers and Warrington, 1982; TeVelde, 1984). Frost has been suggested as limiting corn production in areas that have enough degree days to grow corn successfully, but experience frosts unpredictably or over too long a period of the year (TeVelde, 1984). As an annual crop, corn production would probably not be severely limited by frosts that occur rarely, therefore we do not expect to see INA$^-$ bacteria applied to corn in tropical and subtropical regions. In addition, tropical corn is produced primarily by peasant classes who probably could not afford the INA$^-$ technology. However, the difficulty of seperating out the area of corn growing in tropical regions from world wide statistics precluded our removing these areas in this analysis.

Potatoes are an important food crop. Potato acreage is not exceeding large, being the 8th largest contributor to our estimate of frost vulnerable cropland (Table 5). Potatoes do, however, have some useful characteristics that facilitate exploration of the limitations of our current analysis (see chapter 11).

Should frost become less limiting factor to crop production by any means of INA bacteria control, it is possible that some cropping patterns would shift. Frost sensitive crops might be grown in areas where frost currently limits their production. This would increase the total cropland area on which INA^- bacteria could be applied. This scenario, however, is extremely complicated and would rely on far more information than we can synthesize at this time.

Vulnerable Leaf Area

While leaf area indices vary with crop, time of year, plant spacing, variety, etc., we assume an average LAI of about 3.0 for all frost vulnerable cropland. This value is more typical of annual crops.

Summary

To summarize, our estimate of cropland on which INA^- bacteria might be applied includes about 1.1×10^8 ha in the United States and 7.1×10^8 ha in the world. This is approximately 50% of the world's cultivated land (F.A.O. Production Yearbook, 1985). We estimate a total leaf area of frost vulnerable cropland to be about 3.3×10^8 ha in the United States and 2.1×10^9 ha in the world. These are clearly overestimates. Some cropland that rarely experiences frost damage is included. In addition, we do not consider problems of efficacy and degree of farmer adaption of INA^- bacteria. Instead, we assume that INA^- bacteria are effective, that they are relatively inexpensive, and that they are readily available.

These technical assumptions may not be unreasonable considering analogous, introduced agricultural technologies. For example, pesticides and rhizobium inoculum have both enjoyed widespread acceptance and use over a relatively short period of time. Economic constraints on INA$^-$ bacteria acceptance by farmers are possible. INA$^-$ bacteria may not be cost-effective. However, it is possible that goverment subsidies would decrease the cost of INA$^-$ bacteria in much the same way that pesticides are subsidized in third world countries. If INA$^-$ bacteria are effective at preventing frost nucleation on crop plants, then widespread use on the crops listed in Table 1 could be possible. On the other hand, if INA$^-$ bacteria have limited ability to protect crops against frost, then the question of the potential influence of widespread use of INA$^-$ bacteria on climate becomes moot.

Chapter 5: Natural History and Life Cycles

The natural history and life history of an organism can provide fundamental insight into the biology of that organism. In general, however, relatively little is known about the natural history and life history of bacteria. In this chapter, we review information on the identity, microhabitats, life cycles, and typical interactions of leaf epiphytic bacteria, as a prelude to detailed quantitative discussion of their population biology.

Identity and Microhabitat

There are many species of epiphytic bacteria (Appendix 5). The majority of them are gram-negative and members of the genera: *Erwinia*, *Pseudomonas*, *Xanthomonas*, and *Flavobacterium*; most organisms are not identifiable to species (Blakeman, 1982). Goodfellow et al. (1976) conclude that the phylloplane bacterial community is dominated by only one or two taxa at any one time, but these taxa may vary.

Leaf epiphytes are patchily distributed in natural conditions. They occur along the depressions over the anticlinal walls of epidermal cells (Ruinen, 1961, in Last and Warren, 1972), in depressions between epidermal cells (Leben, 1969), in leaf buds (Leben, et al., 1968), near hydathodes (Frossard, 1981), and associated intact and broken trichomes (Leben, 1969; Schneider and Grogan, 1977). In short, they are commonly found near nutrient or water sources, or in protected areas.

Leaf surfaces are stressful environments for epiphytes. They are subject to rapid fluctuations in relative humidity and temperature, and intense doses of ultraviolet light. Protected sites, such as the inside of a broken trichome, might ameliorate these stresses and allow an epiphytic bacterium to live longer (Blakeman, 1982). Some bacteria can avoid these stressful conditions. When conditions deteriorate, some bacteria may reduce metabolic rate, and enter a relatively inactive state, called hypobiosis (Leben, 1974). The dominant INA species, however, probably do not do this.

Bacteria living on the phylloplane in nature may be morphologically different from bacteria growing on petri dishes. The cell wall of *P. syringae* on leaves in nature is frequently surrounded by another coat of protein/polysaccharides, called a glycocalyx. The function of the glycocalyx is not precisely known, but it might be to enhance survival in natural conditions (Costerton et al., 1981).

Geographic Distribution

INA bacteria are probably distributed world wide (Lindow, 1985a). This is based on two lines of evidence: the sampling of plants for INA bacteria undertaken by Lindow et al. in the United States (1978a), by Makino in Japan (1982) and by Yankofsky et al. (1981) in Israel; and the research done on the distribution of *P. syringae* and *E. herbicola* by plant pathologists interested in the pathogenic strains of *P. syringae* and *E. herbicola*.

P. syringae and *E. herbicola* are ubiquitous and have been found on almost all aerial plant surfaces studied (Lindow, 1983b). Pathovars of *P. syringae* have been found on numerous plant species, including most of the common crop species and trees (Blakeman and Brodie, 1976, Crosse and Garrett, 1963, English and Davis, 1960; Lindow et al., 1978a). Plants with waxy leaves, conifers, some oaks, and some citrus appear to be exceptions, having low or nondetectable *P. syringae* populations (data in Lindow et al., 1978a; Lindow, 1983b). *E. herbicola* is also an ubiquitous epiphyte; it is a very rapid growing yellow pigmented bacteria and can be isolated from a vast array of plant surfaces, acting as a pervasive contaminant in attempts to isolate other micro-organisms (Blakeman and Brodie, 1976; Cross, 1971; Hsieh and Buddenhagen, 1974).

The broad plant species range of the primary INA bacteria, *P. syringae* and *E. herbicola*, indicates that *in vivo* modified INA$^-$ bacteria could be used on a wide range of crops. It also indicates, however, that a large number of naturally growing plants have *P. syringae* and *E. herbicola* populations on them. There is a large natural reservoir of INA bacteria in nature.

INA bacteria are not uniformly distributed geographically. Locally high concentrations occur in different parts of the world. For example Schnell and Vali (1976) found high densities associated with algal blooms at upwellings in the Antarctic Ocean. They (1976) also reported a latitudinal gradient in the concentration of ice nucleating particles in leaf litter, with higher concentrations in northern temperate regions than in the subtropics. Schnell and Tan-Schnell (1982) found tea leaf litter to be a particularly potent source of ice nuclei in Kenya. Thus, population dynamics and spread of INA$^-$ bacteria, and the effects on precipitation may vary regionally. Widescale use of INA$^-$ bacteria in the tropics could potentially have a greater environmental impact than in the northern temperate zone, since there are apparantly fewer INA$^+$ bacteria to displace.

Life History

The details of epiphyte life history in nature are poorly understood. Generation time can be as short as 4 hours for P. syringae, but this is not likely to be sustained for long periods of time in natural conditions. A more reasonable model of the life history of a bacterial epiphyte, such as P. syringae, has environmental conditions alternate between inactive, stressful periods when many bacteria die, and periods favorable for growth and reproduction, such as after rain, dew, or other conditions (Hirano and Upper, unpublished manuscript). Periods of stasis and decline alternate with periods of rapid growth.

In the fall, many bacterial epiphytes will move to the leaf litter with leaf senescence and become part of the bacterial flora associated with decomposing vegetation. Many of these will overwinter there. In some agricultural fields where fall plowing occurs, they will be turned into the soil.

P. syringae, specifically the pathovars syringae and glycinea, can overwinter in dormant plant tissue such as buds (Burr and Katz, 1984; Leben et al., 1968), and seeds (Kendrick and Gardner, 1921; Fryda and Otta, 1978), and on weeds, winter annual crops, and cover crops (Baca and Moore; 1984, Ercolani et al., 1974; Latorre and Jones, 1979; Moore, unpublished manuscript, 1986; Waissbluth and Latorre, 1978). After the snow melted P. syringae populations of 1 x 10^4/gfw occurred on weeds, which were covered by snow. Weeds may be important to P. syringae survival in regions with harsh winter conditions (Ercolani et al., 1974; Latorre and Jones, 1979). In warmer climates, P. syringae survived year round in orchard debris and on all plant surfaces except tree bark. Populations ranged from 0.4 x 10^4 to

2×10^6 cfu per leaf, fruit or blossom (Waissbluth and Latorre, 1978). In some warmer areas of California and Chile, P. syringae populations were higher in the winter and spring, decreasing with the heat and dryness of summer (English and Davis, 1960; Waissbluth and Latorre, 1978).

While P. syringae is a poor colonizer of the soil, it can survive in soil (Daft and Leben, 1973, Graham, 1953). Other researchers found that several strains of P. syringae did not survive in soil for more than a few days (Lindow, 1985b; Lindeman, personal communication, 1986). The more significant overwintering site for P. syringae on annual crops is probably the leaf and plant litter in the soil, not the soil proper. P. syringae successfully overwintered on plant residue either on the soil surface or when incorporated into the soil (Daft and Leben, 1973; Graham, 1953;).

Interactions

The trophic and community interactions of epiphytic bacteria are relatively unknown. Predators and parasites are unreported, except for an artificial system where Bdellovibrio bacteriovora parasitized P. syringae (Scherff, 1973). There are, however, many arthropods that may graze leaf epiphytes.

Epiphytic bacteria appear to be opportunistic feeders. They occur whenever nutrients are being leaked, be it by the leaf (Blakeman, 1982; Frossard, 1981) or by fungal conidia (Blakeman, 1978; 1982; Blakeman and Brodie, 1976). In addition some isolates produce antibiotics (Chakravarti et al., 1972, in Blakeman, 1982). The ecological significance of antibiotic production, however, is generally not known.

Competitive interactions are also poorly understood. E. herbicola is a strong competitor against E. amylovora (Farabee and Lockwood, 1958; Riggle and Klos, 1972), X. campestris, pv. oryzae (Hsieh and Buddenhagen, 1974), and possibly P. syringae (Scherff, 1973). Blakeman (1982) suggests that during resource use, E. herbicola creates acid conditions that inhibit the growth of other pathogens. This, however, is not likely to be the only way epiphytic competition occurs.

Summary

Leaf surfaces are stressful environments, and leaf epiphytes have a variety of ways of avoiding and tolerating these stresses. Epiphytes occur in protected sites on the leaf, some can become metabolically inactive, and many develop a glycocalyx. Growth and reproduction probably occur in spurts after the onset of favorable conditions. These conditions are proceeded and followed by periods of stasis and decline.

P. syringae and E. herbicola are ubiquitous. There is, however, significant geographic variability in their abundance, so local effects of widespread use of INA^- bacteria may be variable.

Virtually nothing is known about the biotic interactions of leaf epiphytes. Predators and parasites are assumed to be virtually non-existant. Despite this, many grazing arthropods, such as mites, collembola, and thrips, must consume many bacteria. Competitive interactions are not understood, except for the unique case of E. herbicola. Finally, a positive response of epiphytes to nutrient availability has been inferred, but these arguments are still tentative. Without this knowledge, many assumptions have been made about how leaf epiphitic bacteria interact or do not interact. These are critical assumptions. In addition, because of differences in morphology, bacteria growing in the field may be very different from laboratory reared organisms. Thus, care must be taken when extrapolating from laboratory results to natural conditons.

Qualitative understanding of bacterial interactions in the phylloplane is important. The interactions determine the structure of any model of population dynamics. For example conceptualization of population dynamics as either a competitive, predator-prey, or diffusion-reaction system restricts the set of possible outcomes. Second, this understanding provides the critical evidence in a quantitative analysis of population dynamics. Knowing how competition occurs when it occurs, can greatly strengthen or severely weaken an argument that two organisms are competitively equivalent.

Chapter 6: Birth and Death

The population dynamics of any organism can be thought of as the result of natality, mortality, immigration, and emigration (Clark et al., 1967). All four processes can be affected by biotic factors, such as competition, predation, and host interactions, and by abiotic factors, such as temperature, humidity, and light. These factors may interact in complex ways to produce any given population dynamic.

In this chapter, we evaluate birth and death processes of leaf epiphytic bacteria on the phylloplane. The goal is to estimate growth rates and determine how varying conditions affect growth rates. In the next chapter we discuss bacterial movement.

Here we emphasize the influence of the habitat on INA^- bacteria population growth. The availability of habitat that is conducive to INA^- bacteria growth will directly influence the probability of INA^- bacteria establishment after deliberate release. Weather conditions, host plant, and interactions among the epiphyte community all contribute to the relative favorability of epiphyte habitat for INA^- bacteria growth and long term survival (Blakeman, 1982; Lindow, 1985a). Population dynamics is manifestly complex, and analysis of bacterial populations is still quite rudimentary.

Methods

Populations of leaf epiphytes have been studied by several different methods. Hirano and Upper (1983) distinguish direct microscopic methods, indirect cultural methods, and a correlative leaf freezing assay for INA bacteria. With regard to quantitative methods, direct microscopy is time consuming and unable to detect small populations. The cultural methods are very sensitive to variation in technique. Removal method (washing, grinding, etc.), vigorousness of removal (duration of washing, intensity of shaking, etc.), receiving solution (with and without surfactants, etc.), dillution technique, and culture medium greatly influence population estimates (Hirano et al., 1982; Hirano and Upper, 1983). Populations are expressed as colony forming units (cfu) per gram fresh or dry weight of tissue, per unit area, or per leaf. While this lack of uniformity in units have been criticized (e.g., Fry and Humphrey, 1978; Parbery et al., 1981), Hirano and Upper (1983) argue that this is probably a less important reason that results cannot be compared between laboratories than technical variability. This is undoubtedly true, but fresh and dry weights of some leaf tissues may vary by an order of magnitude (Parbery et al., 1981). In essence, Hirano and Upper (1983) are saying that sample variability over an order of magnitude is tolerable. All estimates of bacterial population density should be considered relative estimates with at least one order of magnitude uncertainty. This is extremely crude.

Correlative freezing assays estimate INA bacterial densities. The greater is the freezing, the greater is the presumptive density. This method does not distinguish among species of bacteria. Indeed, it does not distinguish among ice nucleating particles. Good correlations between freezing ability and bacterial density (see Hirano and Upper, 1983); but this might not generally occur. Non-viable bacteria are not detected by dillution plate counting, so INA bacterial density can be overestimated.

Abiotic Factors

Growth of *P. syringae* under maximally favorable conditions is rapid. Doubling times *in vivo* of about 3 to 5 hours have been observed for *P. syringae* on cherry trees, corn, and bean under humid conditions; this approaches the doubling times seen *in vitro* (Ercolani and Crosse, 1966; Gross et al., 1983; Hirano and Upper, 1985a).

Harsh, dry conditions appear to prevail on leaf surfaces, making this an environment generally unfavorable for bacteria establishment (Sleesman and Leben, 1976). The microclimate surrounding the plant surfaces probably has an overriding influence on epiphytic bacterial populations. For example, *P. syringae* epiphytic populations increased dramatically after a single rainshower (Hirano et al., 1985). In addition, while susceptible cultivars of bean were more effective sources of inoculum than resistant cultivars for spread of *P. syringae* pv. *phaseolicola*, only when environmental conditions were favorable (sufficient moisture, by rain or irrigation) did the bacterial populations increase and disease symptoms occur (Katherman et al., 1980). Free water on plant surfaces is a key factor influencing epiphytic population growth (Godfrey, 1976, Latorre and Jones, 1979).

Hirano and Upper (1984; in press) studied short term population fluctuations of *P. syringae* and presumptive *P. mesophilica*. Population-density changed several orders of magnitude in a 24 hour period. *P. mesophilica* populations decreased 3-fold during the afternoon, but increased back to its original population density shortly after dewfall. *P. syringae* also showed dramatic diurnal population fluctuations, that were not associated with either dew or rainfall. Populations changed 5-fold or less in most 24 hour periods. However, 28.5-fold and 155-fold changes were also observed.

Conditions for growth and mortality fluctuate dramatically in short time periods. Thus a typical vegetational habitat for P. syringae is a complex mosaic of patches. These patches are highly variable in quality at any particular time, and any one patch might vary dramatically in quality over short, one day periods. Patch quality might vary almost randomly in space and time.

Host Plant Factors

Plants vary dramatically in their suitability to P. syringae growth. INA P. syringae occurred on 78% of 95 plant species tested. Of the plant species with low or non-existent populations of INA P. syringae, a large number had waxy leaves (Lindow et al., 1978). Populations of P. syringae on normal waxy, reduced wax, and glossy (less-wax) mutants of corn were lower on the more waxy plants (Haefele and Lindow, 1984). While other effects of plants on epiphytes are less conclusively demonstrated, their existence is highly likely. For example, tomato leaves contain a protein with in vitro antibacterial activity against P. syringae pv. glycinea (Ersek et al., 1984). In addition, Lindow (1985b) observed that bean and potato plants supported higher populations of both INA and INA$^-$ P. syringae, compared to 20 other plant species tested.

Crop cultivars resistant to P. syringae influence epiphytic population dynamics. Soybean cultivars resistant to P. syringae pv. glycinea had lower epiphytic pseudomonad populations (Mew, 1972) and resistant cherry tree varieties had lower populations of P. syringae

(Crosse, 1959). In an extensive study of host plant response to pathogenic bacteria, Ercolani (1973) found that disease symptoms were induced in susceptable plants when a threshold population was reached. However, when pathogenic bacteria isolates were mixed and injected together into non-hosts, a hypersensitive response appeared quickly, even if population growth was inhibited (Ercolani, 1973). These results imply that there may be some specificity between host plants and their epiphytic bacteria. We conclude that the typical vegetational habitat for P. syringae includes refractory patches, where growth can never occur, and patches that either degrade or aggrade with time.

Leaf age affects certain epiphytes. Plant derived substances that promote or inhibit epiphytic populations may occur in leaf leachates (Godfrey, 1976). Nutrient-rich leachates might be expected to increase with the aging of leaves, and a concomitant increase in pseudmonad populations has been observed. This, however, has not been tested in the field environment (Blakeman and Brodie, 1976). While some investigators have suggested that a succession of epiphytes occurs as the leaf ages, the data are not conclusive.

Host mediated chemotaxis and bacteria mobility might affect bacterial population dynamics. In confined regions, such as might occur on a leaf surface, chemotaxis could allow bacterial populations to increase, when they would not otherwise (Lauffenburger et al., 1982). The importance of chemotaxis, however, remains to be demonstrated. The plant host environment could provide a wide variety of chemotactic substances, including the following attractants: sugars, amino acids, nucleotides, vitamins, and oxygen; and the following repellants: inorganic ions, pH extremes, and some amino acids (Lauffenburger et al., 1982).

Interactions of Epiphytes

Interactions among microorganisms on the leaf surface will also modify the favorability of habitats for INA^{-} bacteria (Lindow, 1985a). Some interactions may be ammensal, with only one organism affected. Competitive interactions have been studied predominantly. Problems to consider in intraspecific and interspecific epiphytic interactions include: 1) the ability of applied INA^{-} bacteria to invade before natural epiphytic populations have established; 2) the ability of applied INA^{-} bacteria to invade when natural epiphytic populations are already established; 3) after establishment, the probability that other species will invade and their influence on the population dynamics of INA^{-} bacteria; and 4) the possible competitive and mutalistic relations among epiphytic populations.

Competition between INA^{-} and INA bacteria.

Intraspecific competition. Intraspecific interaction must be understood to predict the effects of releases of INA^{-} bacteria. The spread of genetically engineered INA^{-} bacteria and the displacement of natural INA bacteria depends critically on the way the two strains interact.

Genetically engineered INA^{-} deletion mutants are very similar to their INA parents. Lindow (1985b) compared the mutants and parents in a vast array of tests. The tests included susceptibility to 23 different antibiotics, metabolism of over a hundred different organic compounds, population dynamics on 22 different plant species and on defined media, and survival in soil during freezing and thawing cycles (Lindow, 1985b). The response of INA^{-} deletion strains were similar in every way to their parent strains under the tested conditions. The only difference was in ice nucleating ability (Lindow, 1985b). Interactions between genetically engineered INA$^-$ bacteria and established epiphytic INA bacteria on crop plants might be expected to resemble other intraspecific interactions.

There are, however, only a limited number of studies on intraspecific interactions of INA and naturally occurring INA$^-$ bacteria. In one of the earlier studies, INA and INA$^-$ *E. herbicola* were grown separately and together, in a chemically specified media and on corn seedlings in the field (Lindow et al., 1983b). Based on an analysis of population growth, the two strains did not interact. This result is surprising because most other work shows that strains interact.

All current evidence indicates that INA$^-$ and INA bacteria are competitive equivalents. INA$^-$ bacteria and INA bacteria of the same species have been described as "co-exisiting" when applied simultaneously in similar doses (Lindow et al., 1983a; Lindemann, personal communication). Competition between INA$^-$ bacteria and INA bacteria is believed to be dose dependent (Lindow, 1985b; Lindow and Connell, 1984; Lindow et al., 1983a) and genetically engineered INA$^-$ bacteria has no apparent competive advantage over parental INA strains (Lindemann, personal communication). Reproductive rates of both strains are approximately the same, and the final population densities are roughly in the same proportions as the starting ones. Thus, by all accounts, INA$^-$ and INA bacteria are competitive equivalents.

It is difficult, however, to see how these studies can be generalized to the natural environment. All of these studies have been conducted under a limited range of laboratory environments. In nature, epiphytic population density can fluctuate widely, and this may have a dramatic impact on the outcome of intraspecific competition.

Some asymmetry in the competitive interaction between INA^+ and INA^- bacteria is indicated; the data, however, are incomplete. Lindemann (unpublished data, Figure 4d) shows evidence that INA^- bacteria may be slightly superior competitors to INA^+ bacteria. While there are no contradictory data, this needs to be more thoroughly investigated.

Invasion. While the rhizosphere can not be compared directly to that of the phylloplane, investigations of interactions between applied and native *Rhizobium* strains of the same species are relevant. Numerous authors have shown that when established *Rhizobium* populations are present in soil, addition of another *Rhizobium* strain of the same species will rarely be competitive at forming nodules on the target host plant (Brockwell et al., 1982). This has been largely attributed to a mass effect, since the population in the soil is often many orders of magnitude larger than what is possible to inoculate with; if soil populations are equal to applied populations then the applied organisms will nodulate at a comparable rate (Amarger and Lobreau, 1982).

Similar, but somewhat contradictory results are seen for phylloplane interactions. Four sets of experiments have been done (Lindow et al., 1983a; Anderson et al., 1984; Lindow, 1985b; Lindemann, unpublished data). Lindow et al.'s (1983a) study used an antagonistic INA^- isolate of *E. herbicola* on field grown corn seedlings. Repeated inoculations with the antagonistic bacteria did not influence established populations (Lindow et al., 1983b). The strains appeared not to interact. In another experiment, antagonistic bacterium strain T7-3 (a naturally occurring INA^- mutant of *P. syringae*), when applied at approximately 10% blossom, colonized almond tissue well. Further, T7-3 grew, migrated to other plant tissue, and reduced colonization of tissue by INA bacteria.

Anderson et al. (1984) conducted a growth chamber experiment involving two different *E. herbicola* strains, one active in ice nucleation and the other a naturally occurring INA^- strain. The antagonist INA^- and the wild type INA strains were inoculated on tomato plants at equal proportions, however, the INA^- strain was inoculated 24 hours before introduction of the INA strain. As measured by the freezing temperature of tomato tissue, the INA^- strain was not given enough of a competitive advantage by the one day earlier inoculation to significantly decrease frost damage (Anderson et al., 1984).

Lindow (1985b) studied invasion of the parent INA *P. syringae* into established populations of INA^- deletion mutants of *P. syringae* (inoculated 48 hours before INA bacteria were added), and found that the invading INA bacteria populations remained about 10^2 cfu per gram of bean leaf. He concluded that INA^- mutants of *P. syringae* may be effective in competitively excluding INA^+ genotypes when high populations of INA^- bacteria can be established. In both the experimental invasion treatment (INA^+ invading established INA^- bacteria) and the control (INA^+ invading uninoculated plants), INA bacteria populations fluctuated about 2 orders of magnitude. The INA isolate that was invading INA^- populations established a low colonizing population, increased, and then decreased. The isolate that was invading uninoculated plants established much higher colonizing populations, decreased, and then increased. Established bacteria may reduce colonization rates of subsequent invaders.

Lindemann (unpublished data), in a series of elegant experiments, has clarified several of the apparant contradictions for INA/INA$^-$ bacterial interactions on the phylloplane. She demonstrated that competition was density dependent. When the dominant bacterial strain reached about 10^7/strawberry blossom, the growth rate of the less common bacterium decreased. Prior to this, growth rates are not affected. This observation unifies the disparate results of Lindow et al. (1983b), Anderson et al. (1984), and Lindow (1985b). Lindow et al. (1983b), observed no interaction between strains; their populations were much lower than Lindemann's 10^7/blossom, and therefore would not be expected to interact. Anderson et al. (1984) found that one day earlier establishment did not suppress invading INA populations; their initial INA$^-$ populations might not have increased high enough to supress INA populations during that limited 24 hour period. Lindow's (1985b) result demonstrated suppression of INA bacteria colonization at high resident population densities.

One competitive model consistent with these data is a site limitation, or carrying capacity model. Assuming that there are only about 10^7 usable sites on a strawberry blossom, or that a strawberry blossom can only support about 10^7 Pseudomonas spp., competition for sites will occur only when total population size nears 10^7. This is different from a simple mass-effect model.

Competition among phylloplane colonists might differ from that of rhizosphere colonists. Native Rhizobium colonize new root tissue rapidly, and quickly pre-empt sites from inoculated Rhizobium strains, unless the inoculated strain is present in large numbers compared to native species (Amarger and Lobreau, 1982). In contrast, large variation in epiphytic bacterial population density occurs on aerial plant surfaces (Lindow et al., 1978, Hsieh and Buddenhagen, 1974, Hirano and Upper, 1984; Hirano et al., 1982; 1985; Waissbluth and Latorre, 1978). Phylloplane colonists could establish on leaf surfaces, even when surrounding plants have high epiphytic populations, because native epiphytes might not colonize new sites fast enough. Colonizable surfaces without established epiphyte competitors might be readily available in most environments. Such colonizable surfaces could be on new plant tissue or on older plant tissue after dramatic weather conditions, such as severe rainstorms (Lindemann and Upper, 1985; Lindow et al., 1983b).

Interspecific Competition. Interspecific epiphytic bacteria competition is also important, however, less is known. The spread and establishment of applied INA$^-$ bacteria in the surrounding environment will be affected by the surrounding epiphytic populations. Interspecific interactions, like intraspecific interactions, might be mediated by a variety of mechanisms, including preemption of favorable habitat sites, scramble for nutrients, altering the environment (e.g., by increasing the acidity), and production of antibiotics (Blakeman, 1982).

The relative ability to survive and regrow after desiccation may be an important aspect of competitive interspecific interactions, particularly in the epiphytic environment (Sleesman and Leben, 1976). Epiphytic survival on plant hosts under harsh, desiccating conditions has not been well studied, although attempts have been made to correlate dry, hot weather with lower populations of *P. syringae*. In a laboratory study, *P.*

syringae and Erwinia carotovora were found to be an order of magnitude less resistant to dessication than Corynebacterium strains (Sleesman and Leben, 1976).

Antibiotic production by bacteria can have an antagonistic influence on other bacteria. P. syringae pv. syringae is a predominant bacterial species of epiphytic populations and, concommitantly, a majority of P. syringae pv. syringae strains produce a wide spectrum of bacteriocins, unlike other epiphytic bacteria (Vidaver, 1976). Some authors suggest that the ability to produce bacteriocins confers a competitive advantage to P. syringae (Vidaver, 1976). Production of antibiotics by E. herbicola has also been found in vitro (Ishimaru and Klos, 1984).

Production of bacteriocins and antibiotics shown under laboratory conditions does not necessarily translate into antagonistic production in the field. Naturally occurring antagonists of INA bacteria, varied in antibiotic production in vitro, but all remained strong antagonists in vivo. This suggests that antibiosis was not important to the relative competiveness of the tested antagonists. There is virtually no conclusive quantitative data on interspecific competition between epiphytes. Interactions between E. herbicola and other species were discussed in chapter 5, but these may be unique. Indeed, given the paucity of data, we assume that interspecific competition is similar to intraspecific competition, at least for the dominant gram-negative taxa.

Spatial Dispersion

Hirano and Upper (Hirano et al., 1982; 1985; Hirano and Upper, in press) demonstrated that numbers of P. syringae are distributed log-normally among sample units. This is true for INA, INA$^-$, and total bacteria, and for populations on corn, soybean, rye, bean, or oats. This appears to be a general phenomenon.

Population densities of INA bacteria typically vary three orders of magnitude or more; they are extremely patchy. This implies that many under-exploited patches occur in any vegetative canopy at any time. We use the published information on bacterial dispersion patterns to estimate the likely frequency of under-exploited patches.

We estimate that relatively small numbers of patches will have low populations of INA bacteria on them (Table 8). When mean population density is low ($10^{3.6}$ on oats), as many as 1/10th of the leaves have fewer than 100 cfu on them. In this case, nearly 2.5 percent of the leaves probably had no INA bacteria on them. When the mean population gets higher, more leaves are occupied, and the proportion of under-exploited leaves becomes small, often less than 0.0001 (Table 8). If the sample unit were smaller, this proportion would be larger.

Table 8. The proportion of sample units with low populations of INA bacteria.

Crop	Bacterium	Mean Density[1] ($\log_{10}$CFU)	Probability of Low populations	Data Source
corn	INA bacteria/gfw 14 Sept. 1979	4.89	.0007	Hirano et al., 1982
Soybean	INA bacteria/gfw	5.2	.000032	Hirano et al., 1982
rye	INA bacteria/gfw	6.4	<0.000001	Hirano et al., 1982
bean	*P. syringae*/leaflet 0900 July 20	5.7	.000108	Hirano and Upper, in press
bean	*P. syringae*/leaflet 0900 July 21	7.3	<0.000001	Hirano and Upper, in press
oats	INA bacteria/leaf	3.6	0.10	Hirano et al., 1985

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[1] read from 50th percentile on graphs or from tabular data.

[2] probability that a sample unit will have 100 or fewer bacteria on it. This was determined by extrapolation of visually fitted regresions on the population density frequency distribution.

We also use these data to estimate the relationship between variance in density and mean density for INA bacteria. The data are not complete enough to do this for total bacterial population density. There are many ways to estimate this relationship (Southwood 1978); we regress the mean density (log cfu) on the variance in density, using the extensive data for corn in Hirano et al. (1982, Table 2). With this relationship, we calculate the probability that a leaf would be under-exploited for any mean density (Figure 2).

There was a relatively poor variance to mean relationship ($n = 6$; $r = -0.58$). This was probably related to the small sample size. Variance appears to increase as mean density decreases. This can be observed in figure 4 of Hirano and Upper (in press); the slope of the rankits is flatter at the lower density. This might not be a general phenomenon, but certainly, variance does not appear to increase with mean density. If the observed relation does in fact hold, then as the overall quality of a habitat increases (higher mean density is sustained), the habitat becomes less variable in quality. Conversely, as the habitat becomes poor, it becomes more variable in quality. Poor habitats have relatively more empty and highly occuppied sites than better habitats. The variance to mean relationship may vary from plant to plant; oats and soybean may be more variable than corn (Figure 2). This result may have important implications for the invasion of introduced bacteria. Invasions of introduced bacteria might be more successful in poorer habitats.

Several models are consistant with the rapid fluctuations in bacterial population density over short periods of time observed by Hirano and Upper (in press). The two extremes might be called a "random environment" model and an "undulating environment" model. In the first, site quality fluctuates randomly from one time period to the next. There is no correlation in relative site quality from one time to the next. The relative and absolute quality might be determined by the environment. In the second model, a poor site is always a poor site relative to the others. Thus, if we were to graph site quality on a spatial array of sites, and observe the resultant surface of site quality, this surface would undulate up and down, but maintain its overall shape. Relative site quality is completely correlated from one time to the next. Relative quality might be completely determined by site characteristics. Clearly the truth will lie somewhere between these, since both site characteristics and the environment will interact. An important consequence is to the extent the environment is random, an invading bacterium landing on an under-exploited site can potentially rapidly grow and attain high population densities. There is a small, non-zero probability that a dispersing bacterium might encounter an under-exploited site and establish a viable population in any habitat.

The situation a dispersing bacterium is likely to encounter in nature can be determined from our Figure 2 and Lindow et al. (1978a) survey data (Figure 3). Using the lower interquartile limits (Figure 3) for INA bacteria (2.6) and total bacteria (4.8), and assuming total bacteria are distributed log normally like INA bacteria on corn, we calculate the proportion of under-exploited sites. For INA bacteria, in one quarter of the habitats at least 24 percent of the sites have fewer than 100 INA bacteria/site. For total bacteria, in one quarter of the habitats, only

0.0067 percent of the sites have fewer than 100 total bacteria/site. In these calculations, a site is about 50 cm^2; for smaller sites, this percentage will be higher. In addition, if the apparently more variable oats or soybean variance to mean relationship were used, the percentages will be much higher. In a typical square meter of vegetation with a LAI = 3, these conditions translate into at least 144 sites with less than 100 INA bacteria/site, and in 100 square meters, at least 4 sites with less than 100 total bacteria on them. These are small numbers, but they could be vastly underestimated.

Growth

Growth rates of epiphytic *P. syringae* have been calculated only by Hirano and Upper (in press) for one day intervals. Other workers have recorded population growth in graphs, but have not calculated population growth rates. We use this published data to estimate growth rates.

Hirano and Upper (in press) sampled bean leaflets in a bean field on a series of consecutive days and reported relative population growth rates (RGR). These can be converted into intrinsic or Malthusian growth rates (r), as $r = \ln (RGR)$. Intrinsic growth rates (r) vary remarkably from day to day, from -1.61 to 5.04 day^{-1} (Table 9).

Table 9. Variation in intrinsic growth rate (r) for P. syringae for different time intervals.

Plant	r^1 (1/day)	Time period^2 (days)	Authority
bean	-1.61 to 1.61	1	Hirano and Upper, in press
bean	3.35	1	Hirano and Upper, in press
bean	5.04	1	Hirano and Upper, in press
bean	0.072	53	Lindow et al., 1978a
soybean	0.067	45	Lindow et al., 1978a
pumpkin	0.044	52	Lindow et al., 1978a
tomato	0.048	62	Lindow et al., 1978a
apple	0.017 to 0.013	60 - 100	Gross et al., 1983
soybean	0.082	22	Kennedy and Ercolani, 1978
peach	0.017	90	Ashworth et al., 1985a

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[1] Calculated for exponential growth model from data published in graphs for long time periods.

[2] Only periods of roughly linear exponential growth were used. Linear portions were determined by eye. Details available upon request.

Intrinsic growth rates can be estimated from graphs of population density versus time; r - log 10 x m, where m is the slope of the graph. Using data in Lindow et al., (1978), Kennedy and Ercolani (1978), Gross et al. (1983), and Ashworth et al. (1985), we estimate growth rates over longer periods of time (Table 9) to vary from 22 to 100 days. Growth rates varied from 0.013 to 0.082 day^{-1}, with a median of 0.048 (Table 9).

These observations may seem to be in contradiction, but the proper picture is that population growth fluctuates wildly from day to day, but steadily grows over long periods of time. This may be because there are more days of positive population growth or that growth is greater on those days. We include both possibilities in our model (chapter 9).

Summary

While host plant and environment dramatically affects the population dynamics of epiphytic bacteria, the ubiquity of INA bacteria indicates that these bacteria have effective means of growth and dispersal. Understanding the effects of the host plant, weather conditions and interspecific and intraspecific interractions on applied INA$^-$ bacteria has only begun to be illuminated. Similarities and differences with rhizobium incolation and applied INA$^-$ bacteria are drawn, and in some cases a mass effect seems to be operating. That is, the timing of inoculation and the quantity of inoculum influence the ability of applied bacteria to grow. However, knowledge about intraspecific and interspecific epiphytic interactions under differing conditions is limited and thus, our conclusions must be tempered. Predation and parasitism is assumed to be generally unimportant. This conclusion certainly needs further testing, but we limit our analysis to a competitive model with environmental fluctuations.

Competition is viewed as a scramble competition for a limited number of sites. The number of possible sites on a unit of habitat fluctuates wildly in time and is highly variable in space. Thus, even in habitats with high mean population densities, there is some non-zero probability of successful colonization, given that a colonist can get there. Typical INA densities range from not detectable to $10^{7.4}$/gfw, with an interquartile range of $10^{2.6}$ to $10^{5.0}$/gfw. Total bacteria density has an interquartile range of $10^{4.8}$ to $10^{6.8}$/gfw. Using our calculated variance to mean ratio we infer that in 25 percent of the potential P. syringae environments, at least 0.24 and 0.0067 percent of the leaf sites have fewer than 100 INA bacteria and total bacteria. These sites are likely candidates for colonization.

The growth rate of P. syringae is highly variable with time, but there are long term trends (Table 9). Daily growth rates usually vary from -1.61 to 1.61, with a maximum of 5.04. Longer term growth rates (22 - 100 days) vary from 0.013 to 0.082 (median = 0.048).

Chapter 7: Dispersal

Dispersal is the remaining component of population dynamics that affects the likelihood of spread of a population. We examine the movement of bacteria on the phylloplane, the mechanisms of take off from the phylloplane, the rate of take off and deposition, survival during dispersal, and transport in the atmosphere. Transport occurs on several spatial scales, and we discuss the local, meso, and continental scales. The goals are to estimate the flux to and from individual sites for colonization in a typical vegetative canopy, to characterize dispersal gradients, and to delineate the major factors affecting dispersal. This information will be used to evaluate the likelihood of spread of introduced INA^- bacteria in the environment.

Movement on the Phylloplane

Movement on the phylloplane has been hypothesized to be of limited range, although many epiphytic bacteria do have flagella and apparently can swim within water films (Blakeman, 1982; Burr and Katz, 1984). Motile *P. syringae* generally occur in nature, although nonmotile mutant strains have been found (Kennedy and Ercolani, 1978). However, movement within plant tissue and on surface water films seems to be less than a centimeter, and clearly is bounded by the area of the plant (Blakeman, 1982; Mew, 1972).

The significance of this movement to our problem is that it implicitly defines the appropriate spatial scale of a "site." Bacteria might be expected to interact on this spatial scale, but not to interact very much when seperated by greater distances. In our discussions thus far (see chapter 6), we have used the published units of gram fresh weight and the area of one leaf or leaflet as an arbitrary size of one site. The actual area of these units varies significantly from one plant species to another, and ranges from 46.7 to 118.4 cm^2/ gfw and from 5.6 to 106.1 cm^2/ leaf (Parbery et al., 1981). For the crop plants that have been studied, these areas might be about 50 cm^2/ leaf and 80 cm^2/ gfw. If bacteria can move about 1 cm on the phylloplane, a "site" would be about 3.1 cm^2, or about 16 sites/ leaf and 26 sites/ gfw. If bacteria are distributed log-normally among this sized site, just like they are among leaves, then we have probably grossly underestimated the occurence of under-exploited sites in a field (chapter 6). This is a critical area where more research is required.

Dispersal Agents and Take Off

Very little is known about how bacteria take off from surfaces (Gregory, 1973). The common epiphytic bacteria probably adhere relatively nondiscriminantly to leaf surfaces. Presumably, the glycocalyx plays an important role in determining how strongly a bacterium adheres (Costerton et al., 1981). This is a layer of polysaccharides and proteins surrounding a bacterial cell wall. In some bacteria it is more developed, and adhesion is stronger when the bacteria are starved (Dawson et al., 1981). If this is a general phenomenon, then adherence might be greater during stationary growth and less during log growth (Kjelleberg et al., 1983; J. Lindemann, personal communication), and so take off from the phylloplane could be dependent on growth rate. While highly speculative, this hypothesis holds a potential advantage for the bacterium; take off that occurred during conditions favorable for bacterial growth might also favor survival in transit and enhance the probability of colonization.

Epiphytic bacteria have a number of dispersal agents and a number of sources from which to originate. Quantitative information on the rate of dispersal by various agents is generally lacking. Anthropogenic agricultural operations that pulverize plant debris or stir soil (Lighthart, 1984; Perombelon et al., 1979) and wind-blown debris (Bovallius et al., 1978) have been found to be major contributors to airborne bacteria. However, the steady flux of epiphytic bacteria upwards during the warm part of the day may be a more significant source of movement of bacteria into the surface layer of air, compared to rare anthropogenic operations such as harvest operations (Lindemann and Upper, 1985). This issue remains unresolved.

In general, rain splash, wind storms, atmospheric stability conditions, agricultural operations, irrigation, insect vectors, and movement of plant debris or seeds, all may play a role in both short and long range movement of epiphytic bacteria (Andow, 1985; Fryda and Otta, 1978; Harrison, 1980; Lindemann, et al., 1979; Lindemann and Upper, 1985; Venette, 1975; Waissbluth and Latorre, 1978). The wide variety of potentially important vectors is illustrated by a study of P. syringae, which was consistently collected from bees, drops of rain, and dew in a year round population and dispersal study in a pear orchard (Waissbluth and Latorre, 1978).

While the number of bacteria moved by insects is likely to be small, this number could be quantitatively important for the spread of a bacterial population. Insects actively search for host plants and encounter large areas of host plant tissue, so any bacterium on an insect has a good probability of encountering a habitat similar to the one it originally was on. Moreover, herbivorous insects typically damage the plants they visit, leaving open wounds that contain high nutrient concentrations. Bacteria moving on insects may be placed in these highly favorable microhabitats. These issues have not been adequately investigated in _P. syringae_.

Survival During Dispersal

Survival of laboratory cultured populations of bacteria during simulated field dispersal is usually poor. For example, exponential mortality rates of _E. herbicola_ were about 0.14 to 0.22/ hour (Southey and Harper, 1971) and of _E. carotovora_ were about the same (Perombelon et al., 1979). These estimates of mortality are likely to be high compared to actual mortality of field populations. Laboratory populations usually lack a glycocalyx, which would protect them from environmental hazards. In addition, actively growing cells, such as in a laboratory culture, are more susceptible to mortality factors than non-growing cells in prophase (Dark and Callow, 1973; Skalig and Eagon, 1972). In fact, it has been suggested that some microorganisms may be able to condense favorable environments around themselves and grow in the atmosphere (Parker, 1984).

Flux to Atmosphere

The flux (or loading or rate of movement) of bacteria from major sources to the atmosphere is not well known. The only quantitative data currently available concern potato haulm pulverizing (Perombelon et al., 1979), grass hay combine operations (Lighthart, 1984), and wet and dry aerosols from plants (Lindemann et al., 1979; 1982; unpublished data;

Lindemann and Upper, 1985; Constantinidou et al., unpublished data).

Little is know about the quantitative effects of wind speed or wind shear stress, except that very high wind speeds and large shear stress generated very high bacterial flux (e.g., Bovallius et al., 1978), and that upward flux from plant canopies occurred only when shearing stresses occurred in the surface boundary layer. These shearing stresses occur only under neutral to unstable atmospheric conditions. Stable atmospheric conditions, when little shear stress occurs, includes cloudless, windless nights and overcast days. Upward flux of bacteria from a bean canopy was about 2.3×10^5 cfu/ha/hour during stable atmospheric conditions, and about 7.0×10^9 cfu/ha/hour in neutral to unstable conditions (Constantinidou et al., unpublished data).

Upward flux from potato haulm pulverizing was about 2.2×10^8 cfu/ ha·operation, which was about 1×10^{-4} to 1×10^{-5} of the total bacteria population on the potatoes (Perombelon et al., 1979).

Lighthart (1984) estimated the bacterial loading from grass/hay combining using a solution to Pasquill's equation for turbulent diffusion. He calculated that aerial concentrations at the combine source must be about 6.4×10^8 cfu/ m^3. If the wind speed were 1 to 10 m/sec, then flux would be between 6.4×10^8 to 6.4×10^9 cfu/ m^2/ sec or 2.3×10^{12} to 2.3×10^{13} cfu/ m^2/ hour. These are extremely high values.

The most complete studies on bacterial flux have been done at Wisconsin (Lindemann et al., 1980; 1982; unpublished data; Lindemann and Upper, 1985; Constantinidou et al., unpublished data). Using their data for total bacteria (Lindemann et al., 1980; 1982; unpublished data), we regressed leaf surface density on flux, and calculated that log flux (cfu/

m^2 sec) = 0.48 + 0.26 log leaf density (cfu/gfw) ($n = 7$, $r = 0.666$). These data are for several plant species with varying canopy structures and specific leaf weights, and for different dates with different wind speeds (all dates, however, were ones with unstable to neutral atmospheric conditions). One might have expected a poorer relation with such a small sample size. This provides tentative quantitative evidence that flux is proportional to population density on the phylloplane. If LAI= 3 and 1 gfw = 50 cm^2, then between 3.6×10^{-3} and 4.0×10^{-6} of the total bacteria move from the phylloplane into the atmosphere per hour. Median flux from a more or less complete bean canopy was about 1,100 cfu/ leaflet/ hour (assuming 1 leaflet = 50 cm^2 and LAI= 3) (Lindemann and Upper, 1985). Median flux was lower from small plots, 370 and 450 cfu/ leaflet/ hour (1981,1982), than for larger plots, 1,500 cfu/ leaflet/ hour (1980) (Lindemann et al., unpublished data).

Bacterial flux is dramatically affected by rain. Upward movement of phylloplane bacteria increased some during rain (rain, 115 cfu/ m^2 sec compared to 61 cfu/ m^2 sec; Lindemann and Upper, 1985; Lindemann et al., unpublished data), and downward movement increased dramatically (see deposition, below). Movement from leaves to soil was about 10^5 cfu/ leaflet in a 15 minute rain of 0.4 cm/ hour (Lindemann and Upper, 1985). The net effect of rain was 1) to redistribute phylloplane bacteria in the field, 2) to add bacterial colonists, 3) to reduce leaf population density, and 4) probably to create conditions favorable to bacterial growth.

Transport

Wind may play an important role for airborne dispersal within the vegetative layer. Examples of wind dispersed bacteria include: *Erwinia amylovora*, *Xanthomonas* spp., and *Bacillus* spp. (see Andow, 1985). Wind blown soil, and plant debris with associated bacteria were thought to contribute to the spread of these diseases. Windblown rain has been suggested as an important means of transmitting the following phytopathogenic bacteria: *Erwinia amylovora*, *P. syringae* pv. *glycinea*, *P. syringae* pv. *morsprunorum*, *P. syringae* pv. *phaseolicola*, *Xanthomonas* spp., *Corynebacterium* spp., and INA bacteria (Butcher et al., 1967; Daft and Leben, 1972; Ercolani et al., 1974; Lindemann and Upper, 1985; Stevens et al., 1918; Venette, 1975).

Aerial transport can be thought of as occurring on several spatial scales within a vertically structured environment. The heights in which most transport occurs is in the planetary surface boundary layer, which is defined to be the layer of air from the planet surface to the air temperature inversion. This layer varies dramatically in thickness from a few meters to several hundred meters, depending on wind speed, cloud cover, and several other factors (Pedgley, 1982). Many airborne particles make it above the planetary boundary layer and are moved in different air systems, such as the jet streams. Almost no particles are found in the stratosphere (Hobbs and Yates, 1985).

Transport processes have been studied on at least five spatial scales, micro-, local, meso-, continental, and global. Investigations at the micro-scale typically examine within field movement of particles, frequently focusing on scales of several meters and time spans of a few hours to days. Transport models that are reasonable approximations to these processes are frequently very similar to passive diffusion models (see for example, Okubo, 1981). In these models, the environment is characterized as homogeneous and movement occurs by many random displacements. At the local scale, investigations have focussed on movement between fields, on the order of 100's of meters and weeks. The Gaussian plume model has proven to be satisfactory for this scale (see for example, Pasquill, 1971). In this model, the planetary boundary layer is recognized as a bounded, homogeneous environment, and dispersal occurs in a uniform windfield with homogeneous turbulence. This model has the most use for modeling atmospheric transport, and has proven satisfactory in applications in plant pathology (Kampmeijer and Zadoks, 1977).

The global scale considers long time scales and the entire earth. In most of the global models, it is assumed that conditions result in thorough mixing in the atmosphere and the process is determined primarily by loading and deposition rates on the earth's surface. In other words, the atmosphere is considered to be an enormous pool into which particles go, and out of which particles come, and the detailed structure of the atmosphere plays an insignificant role in either the process or the effects under consideration. The meso-scale and continental scale have received relatively less research attention. The distinction between these to scales blurs somewhat because they have not been well characterized. Investigations on the continental scale examine movement over 100's of kilometers. The models are of the Lagrangian trajectory type, and assume that vertical mixing is complete (Eliassen, 1978; Young, 1982). Attention can then be focused entirely on horizontal processes with realistic detail. Movement on the meso-scale occurs on the order of 1000's of meters, again over arbitrary lengths of time. At this scale, it is not possible to ignore diurnal variation in the height of the inversion layer and vertical mixing, and it is necessary to allow spatial variation in the windfield and turbulence (van Dop and de Haan, 1984). This is the most difficult scale to model, and work has just been initiated. Most studies have been empirical.

While little work has been done on the dispersal of plant pathogens at each of these scales (except for work on wheat rust), and virtually none done on the dispersal of epiphytic bacteria, there are three lines of evidence that more than just micro-scale dispersal occurs and that dispersal may occur on very large spatial scales.

Constantinidou et al. (unpublished data) compared the ice nucleation activity and the phytopathogenic host range of P. syringae that were resident in a bean canopy and P. syringae that were entering the bean canopy from the atmosphere. They found significant differences in both characters between the two populations. This implied that the source of the incoming aerosol could not have been the bean field that they were studying, so dispersal must occur on a larger than micro-scale.

Bovallius et al. (1980) reviewed evidence that there is long range movement of bacteria. They cited one example where long range dispersal clearly had occurred (Bovallius et al., 1978). The occurence of bacteria at high altitudes and distant locations, led the authers to conclude that long distance movement of bacteria is a wide spread phenomenon. Several examples of phytopathogenic bacteria, where aerial transport over long distances is apparantly an important means of disease transmission, indicates that epiphytic bacteria might be involved in long-range transport in the atmosphere (Akers, et al., 1979; Quinn et al., 1980).

The third line of evidence is provided by Selander and his colleagues (Ochman and Selander, 1984; Ochman et al., 1983; Selander and Levin, 1980; Selander et al., 1985; Whittam et al., 1983). They characterized the genetic structure of populations of *Legionella pneumophila* and *Escherischia coli* using isoenzyme analysis. *L. pneumophila* typically is associated with the phycosphere of fresh water algae (Cole, 1982), but is also commonly found in hospitals (it is the pathogen of Legionaire's disease), and *E. coli* is an inhabitant of the gut. They found that the genetic structure was consistant with a model of a haploid clonal species. In addition, they found that similar genotypes occurred in widely different localities, and suggested that these genotypes were probably derived from a common ancestor. This implies that long distance dispersal frequently occurs in these species. Since there is nothing particularly remarkable about the ecology of these two species to distinguish them from leaf epiphytes, we expect that *P. syringae* can disperse long distances too.

Limited data are available on long-range dispersal of bacteria, so studies on the dispersal of other particles are of interest, particularly particles that are similar in size to bacteria. Bacteria can vary tremendously in length, from approximately 0.3 um to 15 um (Akers, et al., 1979). Smoke plume experiments, where smoke is used as a tracer to investigate dispersal in the atmosphere, have been used to gain information about dispersal of particles similar in size to bacteria (Mikkelsen and Eckman, 1985, Pedgley, 1982). Measuring the affects of wind and rain on dispersal of smoke plumes provides data that is greatly needed for attempts to model meteorological processes and dispersal of clouds of small organisms (Mikkelsen and Eckman, 1985, Pedgley, 1982). Similarly, observations on the dispersal of pollen plumes, under varying conditions, can increase our understanding of short-range and long-range bacteria dispersal (Pedgley, 1982).

Local scale movement. The local scale movement of plant pathogens has been well studied. Dispersal and disease gradients have been measured for many pathogens (Gregory, 1973). Rather than review all the factors that influence dispersal gradients, we simply characterize their shape and develop a quantitative relationship between dispersal and distance. Two empirical equations have been used to describe gradient shape from a source (see McCartney and Fitt, 1985), a power law relationship and an exponential decay relationship. It is not yet clear which provides the better description, but neither have good physical analogies. The most robust similarity among these two models and the Gaussian plume model, which is based on turbulant diffusion, is that the dispersal-distance relationship is leptokurtic. In other words, compared to a normal distribution of dispersal distances, observed dispersal exhibits a greater tendency to go far distances. Thus any model that relies on normally distributed dispersal will underestimate the potential for spread of the microbe. This is particularly relevant for our problem because the theory that has been developed to understand spread is well characterized for normally distributed dispersal, and barely understood for leptokurtic dispersal.

Typically, measured dispersal gradients show great variability (vanderPlank, 1967). We calculate the median dispersal distance for a wide variety of plant pathogens, using the data that were summarized in

McCartney and Fitt (1985) and Fitt and McCartney (in press) for both the power law and the exponential relationship. These distances varied from 0.04 m to 87 m. Leaf epiphytes will probably fall somewhere in this range.

Lighthart and Frisch (1976) developed a dispersal model based on the Gaussian plume model of turbulent diffusion. This model has been shown to provide good predictions for bacterial dispersal on a local scale (Perombelon et al., 1979). Lighthart and Frisch (1976) predicted dispersal as a function of release height, release rate, wind speed, atmospheric stability class, and mortality during dispersal. We generated dispersal predictions for epiphytic bacteria, and found that for the range of bacterial survival observed (mortality constant near 0.1/ hour), variation in survival had little effect on predicted dispersal. However, the greater the release rate and the more unstable the atmosphere, the greater was the dispersal distance. Release height affected dispersal only for unstable atmospheric classes.

The shape of a plume of dispersing organisms is likely to be long and narrow, just like smoke plumes (Mikkelsen and Eckman, 1985). This implies that concentrations of organisms passing over any particular site are likely to be either present in reasonably high concentrations or completely absent (Hanna, 1984). Thus, successful dispersal is likely to be extremely heterogeneous.

Meso-scale movement. There are few data on meso-scale dispersal, however, studies on pollen dispersal characterize the form of meso-scale dispersal. Solomon and Silkworth (1986) showed that pollen dispersal fell off rapidly from the source over distances of a few kilometers, just as would be predicted from the Gaussian plume model. Their more significant finding for our discussion was that the pollen also dispersed widely, covering 10's of kilometers with a uniform, low concentration. Interestingly, this concentration was only a few orders of magnitude smaller than the concentrations recovered near the source. If this is a general phenomenon, it would be significant for modeling the spread of a bacterium.

Continental scale movement. Continental scale movements are best thought of as discrete events. They are generally associated with particular weather systems, and movement occurs by great leaps forward, not by the slow accumulation of many small steps. The only documented example of continental scale movement by a bacterium was reported by Bovallius et al. (1978). They showed that Bacillus spp. moved from the Black Sea to Sweden in air currents above the surface boundary layer. The effectiveness of this kind of dispersal is related to higher concentrations at the source, lower mortality during dispersal (At larger spatial scales, mortality during dispersal becomes important. This is because long distance dispersal takes much more time than local dispersal.), and lower wash-out rates from the atmosphere (Fisher, 1984). In addition, transport could occur both in the jet streams above the boundary layer and in the weather fronts within the boundary layer. As a complicating factor, it has been argued that the average behavior of the atmosphere at continental scales yields little useful information regarding the movement and mixing of particles (Hosler, 1963; Hobbs and Yates, 1985). The particular behavior matters more. Thus, an appropriate model for the spread of a bacterium at this spatial scale might be one that characterizes the environment as a series of discrete patches of randomly varying quality connected by a stochastic dispersal process.

Deposition

Deposition of bacteria onto leaf surfaces appears to occur continuously. Deposition rates have been measured during the morning, afternoon and night, and during dry and wet weather (Lindemann and Upper, 1975; Lindemann et al., unpublished data). No diurnal pattern has yet been suggested, but deposition is much larger during rainstorms (Lindemann and Upper, 1985; Constantinidou et al., unpublished data). Over 30 percent of the total atmospheric dust near the planetary surface washed out daily with precipitation (Gregory, 1973). Dry deposition of total bacteria occurred at 15 cfu/ leaflet/ hour (Lindeman and Upper, 1985), 20 cfu/ leaflet/ hour (Constantinidou et al., unpublished data), and 47 cfu/ leaflet/ hour (Lindemann et al., unpublished data). Deposition of total bacteria during rain occurred at 4200 cfu/ leaflet/ hour (Lindemann and Upper, 1985) and 740 cfu/ leaflet/ hour (Constantinidou et al., unpublished data), and may vary with the intensity and duration of the rain.

Survival of the bacteria after deposition on a leaf is probably high during wet deposition. During dry deposition, however, survival of some bacteria has been reported to be poor (Surico et al., 1981). It is unclear if these phenomena are robust generalities.

Summary

Movement of P. syringae on the phylloplane probably occurs at most on a small scale of 1 cm. This implies that bacteria on a leaf might not interact if they are far enough apart, and that our concept of a "site" in chapter 6 (50 cm^2) is much too large. We may be grossly underestimating the occurence of under-exploited sites in the field (chapter 6). We will, however, proceed using the larger "site" in our analysis in Chapter 9.

Dispersal agents of P. syringae are not well characterized. Wind and rain splash are important agents, and similar to other bacteria, anthropogenic activities and insects may be important agents. We focus on the first three.

There are few studies on survival of naturally occurring bacteria during dispersal. Most studies use laboratory grown bacteria.

The movement of P. syringae from leaf surfaces into the atmosphere could occur during mechanical operations in agriculture and on dry days. Potato haulm pulverizing can project 2.2 x 10^8 cfu/ha of bacteria into the atmosphere, hay combining can project 2.3 x 10^{12} cfu/machine hour, and in neutral to unstable atmospheric conditions, about 7.0 x 10^9 cfu/ha/hour can be projected. The greater the epiphytic density, and the larger the agricultural field, the greater was the movement of P. syringae into the atmosphere. Rain redistributed bacteria in a field, added colonists, reduced local population density, and probably created conditions favorable for bacterial growth.

Focusing on wind transport, we examine transport on five spatial scales: micro, local, meso, continental, and global. The mathematical models most appropriate for these scales might be: micro -- passive diffusion; local -- turbulent diffusion/Gaussian Plume Model; meso -- patches of habitat with uniform dispersal among patches; continental -- patches of habitat of randomly varying quality connected by a stochastic dispersal process; global -- no transport model is needed. Long distance dispersal probably occurs at significant rates.

Deposition of bacteria from the atmosphere to the leaf is much higher during rain (740-4200 cfu/50 cm^2/hour) than during dry periods (15-47 cfu/50cm^2/hour). Bacterial survival is likely to be higher during wet deposition than during dry deposition.

Chapter 8: INA Adaptive Value

To evaluate the likelihood of spread and displacement of INA bacteria by INA^- bacteria, it is necessary to ask if INA bacteria have any advantages over INA^- bacteria. Four possibilities have been suggested: 1) there are no significant differences between INA and INA^- bacteria, 2) the INA^+ phenotype confers enhanced pathogenicity, 3) the INA^+ phenotype enhances dispersal, and 4) other possibilities, such as poorer growth by INA^+ phenotypes, unknown growth advantages, etc. There is evidence for all four positions.

One important piece of information to keep in mind during this discussion is the relative gene frequencies of INA^+ and INA^-. If we assume that Table 1 is representative of gene frequencies, the frequency of INA^+ in *P. syringae* is about 0.545, in *P. fluorescens* about 0.25, in *P. viridiflava* about 0.42, and in *E. herbicola* about 0.05. Our discussion of adaptive value should account for the commonness of INA^+ in *P. syringae* and the variation among species. In general, however, the selection hypotheses are too simple to do this without generating a large number of *ad hoc* biological hypotheses. While this would make an excellent research program, we mention it here to dampen enthusiastic acceptance of any one of our possibilities.

Neutral phenotype

This is a widely held view by many of the workers who have examined INA^-/INA^+ bacterial competition. The lack of competitive exclusion and the apparent competitive equivalence of the two types support this view (Lindow et al., 1978c; Lindemann, unpublished data).

This is not an untenuous position. Even though the INA^+ phenotype is clearly different from INA^-, and its effects have different consequences for the organism, there are theoretical arguments supporting this position.

There is an important distinction between genotype and phenotype. Selection acts on the phenotype and evolution occurs only when genotypic frequencies change (Lewontin, 1964). The INA^+ phenotype is variably expressed among individuals with putative INA^+ genotypes, and many environmental factors influence gene expression (Lindow et al., 1982b). Because of this and because several bacterial genomes have high levels of linkage disequillibrium (Selander, 1985), selection at other loci may negate what little selective advantage INA^+ confers.

Of the two bacteria where genetic structure has been intensively studied, there is no evidence for strong selection acting on either of the species (Selander, 1985). The two species were pathogenic strains of _Escherichia coli_ and _Legionella pneumophila_. Both species might be expected to exhibit evidence of clonal selection for some character. Thus, the neutral gene model may hold overall for a bacteria species, despite occurrance of locally intense selection. The consequences of this model are developed in the next chapter.

Enhanced Pathogenicity

The role of ice nucleation activity in association with pathogenicity is particularly intriguing. If ice nucleation activity confers an advantage, then a large porportion of _P. syringae_, _E. herbicola_ and other epiphytic bacteria might be expected to be INA strains, rather than the relatively rare and variable phenomenon it is. This leads us to infer either that ice nucleation activity is a fairly recent adaption, or that the INA genotype confers a competitive advantage only under specific conditions.

The well established association between INA bacteria strains and plant frost injury (Arny et al., 1976; deKam, 1982; Klement et al., 1984; Lindow, 1983b; Lindow et al., 1982a; Lindow and Connell, 1984; Panagopoulos and Crosse, 1964; Weaver, 1981) suggests a role for ice nucleation activity in enhanced pathogencity. Epiphytic populations are dependent on nutrients supplied by the plant host. A mechanism, such as ice nucleation, that increased frost injury, might augment nutrient supplies locally (from injured plant cells), and allow rapid reproduction (Lindow, 1983b). This might not result in any particular selective advantage, since other epiphytic organisms would also be in a position to use the nutrients released by ice ruptured plant cells. However, the adaptive value of ice nucleating activity only has to occur on average in order to confer a selective advantage. Examples show that immediately following frost injury in three different tree species, dramatic increases in P. syringae populations occurred (deKam, 1982; Klement et al., 1984; Panagopoulos and Crosse, 1964). Thus, there is evidence that after inducing freezing INA bacteria can rapidly increase. Ice nucleation activity also has the possible disadvantage of increasing plant senescence in annuals, with a concomitant decline in epiphytic populations.

While a causual relationship between ice nucleation active phytopathogenic pseudomonads and frost injury can be shown (Klement et al., 1984; Lindow et al., 1978c; Lindow, 1985a; Lindow and Connell, 1984), the relationship between increased pathogenicity and increased frost injury caused by INA bacteria is not as clear (Kennedy, personal communication). An association between frost injury and pseudomonad populations was shown by Panagopoulos and Crosse (1964). They found that frost injury was followed by an increase in pathogenic pseudonomad populations. Similarly, in an elegant study, Klement et al. (1984) demonstrated that bacterial canker in apricot trees occurred only when trees were initially damaged by frost and bacteria (P. syringae) colonized and reproduced in the injury. Other workers have reported similar findings in poplar (deKam, 1982) and several other woody plants (Canfield et al., in press).

Some herbaceous plants suffered enhanced frost injury when INA bacteria populations were artificially increased or naturally higher (Arny et al., 1976; Ashworth et al., 1985b; Lindow, 1982b; Lindow et al., 1982b; Lindow, 1985a). In tree crops, frost injury was decreased by inhibiting INA bacteria on almond (Lindow and Connell, 1985), however frost injury was not correlated with INA bacteria populations in other orchard situations (Gross et al., 1984; Ashworth and Davis, 1984). None of these studies investigated the long term effect of increased frost injury on INA bacteria survival and pathogencity. Thus, an association between populations of INA bacteria and frost injury can be shown in many cases, but whether this association increases the pathogencity or the overall growth rate of the bacteria is difficult to determine.

Enhanced Disperal

Ice nucleation activity has also been hypothesized to enhance dispersal and survival of INA bacteria (Sands et al., 1982). INA bacteria will form ice crystals around themselves in the upper atmosphere, and become larger particles. This gives the bacteria a more effective means of leaving the atmosphere (Caple, personal communication). Furthermore, arrival on a plant surface would generally occur during conditions favorable for bacteria growth and survival, that is, rainfall.

The movement of bacteria into the atmosphere and the long range transport of bacteria in the atmosphere, however, is poorly understood (Bovallius et al., 1980; see also chapter 7). If long distance dispersal of epiphytic bacteria is an important aspect of the life cycle of many epiphytic bacteria, as has been suggested for Erwinia soft rot bacteria (Quinn et al., 1980), then differential dispersal could be ecologically and evolutionarily significant. Rain washed leaves are considered prime locations for relatively high rates of epiphytic bacterial establishment (Lindemann and Upper, 1985). Resident bacterial populations could be reduced by rain washing and a wet environment will allow maximal population growth of the incoming bacteria.

A critical question is the relative effectiveness of other ways bacteria leave the atmosphere, and any survival benefit confered by moving out of the atmosphere at the center of a raindrop. If, as Pedgley (1982) suggests, the small size of bacteria poses a particular problem for the washing out from the upper atmosphere, then one might expect that ice nucleation activity could considerably increase the movement of bacteria out of the atmosphere. Pruppacher and Klett (1979) in contrast, consider the capture of bacteria-sized particles by falling raindrops as sufficient to account for quantities detected in raindrops. Pedgley (1982) argues that most bacteria will stream around falling condensation nuclei when the water droplets are small enough. Clearly, once particles become large, many bacteria are absorbed by the moving particles (Mandrioli et al., 1973), and near the canopy nearly all airborne particles are scrubbed from the atmosphere (Gregory, 1973; Constantinidou et al., unpublished data). Raindrops formed from nucleated INA bacteria appear to have the potential to move bacteria efficiently from the cloud layer to plant surfaces. However, this role of ice nucleation activity in bacteria dispersal has only begun to be investigated. Much further research is needed before conclusions about this possible ecological function of ice nucleation activity in INA bacteria can be made.

Other Advantages and Disadvantages

INA$^+$ phenotypes may be at a physiological disadvantage because of the metabolic costs of producing the ice nucleating membrane complex. These types of physiological and metabolic costs, while widely invoked in evolutionary thinking, are frequently small or insignificant. For INA$^+$ phenotypes, the relevant details are unknown.

INA$^+$ phenotypes may confer other growth advantages related to temperature, humidity, leaf age, etc. INA bacterial population densities sometimes fluctuate differently than similar INA$^-$ bacteria (see for example, Lindow et al., 1978a; Gross et al., 1983). Cold conditioning increased nucleation activity in some strains of P. syringae (Anderson et al., 1982).

If there is an advantage or disadvantage to the INA$^+$ phenotype, it is likely to involve a complicated interaction between bacterial population parameters and the environment. Our current understanding is rudimentary, and research on this question is likely to be productive.

Summary

Ice nucleation activity may provide a competitive advantage to INA bacteria in certain situations, possibly by allowing increased population growth by conditioning the resource, or by increasing the efficiency of atmospheric dispersal. Released INA^- bacteria would not have either of these advantages, which would potentially decrease the ability of this organism to spread and establish. However these advantages might be offset by the continual release of INA^- bacteria, which would provide an artificial advantage to the IN^- genotype. This type of advantage could be particularly important if INA^+ and INA^- were selectively neutral.

Chapter 9. Spread.

The spread of an organism should be distinguished from its dispersal. Dispersal occurs to *individuals*, and is the movement of individuals. Spread occurs to *populations* and describes the process of range expansion of a genotype, species, or population. Spread models were first introduced to biology in the context of epidemics (Brownlee, 1911), but the first mathematical expression of spread was for the spread of a rare genotype (Fisher, 1937). Kolmogorov et al. (1937) gave a general solution to the problem posed by Fisher, and Skellam (1951) introduced the model to ecological contexts. Okubo (1980) provides a useful summary of this work, which rests on various modifications of diffusion-reaction equations. In another context Kendall (1948) developed a more general model for spread, which is called the spatial contact model (Mollison, 1977). The literature on the spread of organisms is rich in both theoretical and empirical results, and we draw on these models to analyse the spread of INA$^-$ bacteria.

If INA$^-$ bacteria were extremely unlikely to spread, then the potential impact of widescale release of INA$^-$ bacteria for frost prevention in agriculture would be limited. Once the INA$^-$ bacteria were no longer released their populations could only be invaded by naturally occurring INA bacteria. Thus, their populations must decrease, and the problem is limited both in space and in time. We focus on the potential for spread of INA$^-$ bacteria and displacement of indigenous INA bacteria.

In this chapter, we summarize our findings from chapters 6 and 7 to formulate a series of models for the spread of INA$^-$ bacteria. These formulations vary with spatial scale. When possible, we use the quantitative data to parameterize these models, and explore the implications of the models. Finally we develop competition models, and explore the dynamics of spatially distributed competition models. The goal is to estimate the likelihood that large releases of INA⁻ bacteria will shift the composition of INA bacterial populations.

In this discussion, we focus attention on the dynamics of P. syringae; it is the most abundant INA bacterial species known. We presume that P. syringae is primarily a leaf epiphyte and will not be found in decaying organic matter. This assumption increases the likelihood that INA⁻ bacteria can displace INA bacteria, since there would be fewer natural INA bacteria to displace. P. syringae may, in fact, be present in significant numbers in the soil. It has been shown to survive associated with plant debris over the winter (Daft and Leben, 1972), and has been isolated from debris derived INA bacteria (Schnell and Tan-Schnell, 1982; Schnell et al., 1981). This issue is critical and deserves more work.

Our strategy is to examine both local and global spread. We believe that spread must occur locally before it occurs globally, but the global model provides insight into a worst case scenario over a long time period. We do not, however, develop an explicit global model.

Models of Spread

For spread at the local scale, the environment resembles a series of spatially distributed patches ("sites" in chapter 6 and 7) that vary randomly in quality, both spatially and temporally. Growth by the bacteria is uneven, generally varying between -1.6 to 1.6 day^{-1}. Dispersal could be modelled as a spatial contact process, which allows for leptokurtic dispersal. From Mollison's (1977) work, however, we know that qualitatively, spatial contact models behave like passive diffusion models. We use the diffusion model for qualitative understanding of the local spread problem. It should be borne in mind that the passive diffusion model underestimates the likelihood of spread compared to the more general spatial contact model. We have developed a simulation model for spread in defined habitats, which is based on Kampmeijer and Zadoks (1977) EPIMUL, a plant disease epidemic simulator, and have used the parameters estimated in the previous chapters to simulate spread of INA^- bacteria.

At the meso-scale, the environment is composed of a number of patches, with no explicit spatial relation to each other. These patches, which should be thought of as fields of crops, stands of woods, and blocks of meadows and pastures, vary in their susceptibility to invasion, because the proportion of under-exploited sites varies in each habitat. This proportion will fluctuate somewhat in time, but patch to patch variability could be maintained because of differences in vegetation composition. On this larger spatial scale the minute fluctuations from site to site and from hour to hour become averaged and more global properties become more important. Dispersal can be considered to be a constant low level input into each patch, that varies with the density of potential dispersers in all patches.

At the continental scale, the environment may be best characterized as randomly varying with respect to the probability of successful invasion. On this scale, however, dispersal, i.e. invasion events, resembles a discrete stochastic process, occurring infrequently between patches.

In all of these models, population growth within a patch should be logistic. The maximum population density that the introduced bacteria can attain at the local scale will depend on how many bacteria are already present. The maxima for the other scales will depend on the results for the finer spatial scales.

Global Argument

If we assume that there are no selective advantages to either INA^- or INA^+ phenotypes in a population, then the process of global spread of the INA^- genotype can be analysed as random genetic drift in neutral gene theory (see Crow and Kimura, 1970). This theory states that genetic diversity in a population is determined by mutation rates and effective population size. The greater is the population size, the more genetic variability will be preserved. Assuming a constant mutation rate, the expected distribution of gene frequencies in a given population size can be calculated (Crow and Kimura, 1974). If, however, the mutation rate for one genotype is much higher than the others, then, this genotype will tend to dominate the others.

Widespread release of INA^- bacteria into the natural population can be thought of as a high mutation rate for INA^- genotypes. If INA^- bacteria were applied to 7.1×10^8 ha/ year, and these bacteria multiplied to 10^8 cfu/ leaflet, then 4.26×10^{23} INA^- bacteria may appear from antropogenic sources per year. If the area of all other vegetation that could support equivalent population densities of INA bacteria were 100 times this, and mutation rates of INA^- to INA^+ were 10^{-5}, then the number of natural mutations would be about 3 orders of magnitude less (4.26×10^{20}) than the release rate. Thus, "mutation" rates from INA^+ to INA^- could be as high as

10^{-2}. Since the population density of leaf epiphytic bacteria fluctuate wildly during the growing season by several orders of magnitude and decrease to very small populations during the winter, the effective population size of these bacteria may be much less than their average. If we assume that bacterial populations decrease to population size one billionth their peak size, effective population size would still be over 4.26×10^{16}. For our purposes this can be considered to be an infinite population size.

Three questions can be answered using the theory of population genetics: 1) What is the expected equilibrium frequency of INA^- genotypes under conditions of widescale release? 2) What is the probability that INA^- bacteria will eliminate INA bacteria? 3) How long would it take for INA^- bacteria to eliminate INA bacteria?

For infinite populations under mutation pressure with back-mutation, the equilibrium frequency of an allele is related to its mutation rate. For two alleles with a forward mutation rate of u_1, to allele A_1, and a back mutation rate of u_2 to allele A_2, the equilibrium frequency of A_1 equals $u_1/(u_1 + u_2)$ (Wright, 1949; Crow and Kimura, 1970, pp. 262, ff.). For our case with $A_1 = INA^-$ and $A_2 = INA^+$, $u_1 = 10^{-2}$ and $u_2 = 10^{-5}$. We calculate the equilibrium gene frequencies for INA^- under different levels of agricultural release, using the highest possible release rate and two lower, more probable release rates (see Chapter 11). The equilibrium frequency of INA^- genotypes does not change much with release rate, as long as the release rate is an order of magnitude higher than the natural back-mutation rate (Table 10). The equilibrium frequency varies from 0.91 to 0.99.

Table 10. Predicted equilibrium frequency, probability of fixation, and half-time to equilibrium of INA^- bacteria in nature, assuming INA^- and INA genotypes are selectively neutral. See text for further explanation.

Release rate		1×10^{-2}	1×10^{-3}	1×10^{-4}
Equilibrium frequency of INA^-		0.999	0.990	0.909
Probability of fixation of INA^-	1 year	0.010	0.001	0.0001
	10 years	0.096	0.010	0.001
	50 years	0.395	0.050	0.005
Half time to equillibrium (single release) (years)		69	690	6300

The probability that INA^- bacteria would eliminate INA bacteria in nature is the same as the probability that the INA^- genotype is fixed in the bacterial population. This problem was considered in great detail by Kimura (1957, 1962). We use some of his more elementary results (Crow and Kimura, 1970). When neither allele has a selective advantage, the probability of fixation of an allele is simply p, the proportion that allele is of the total population. For INA^- bacteria applied to agricultural crops, p could be as large as 10^{-2} for a single year. If each year is independent of the others, and similar amounts of INA^- bacteria are released in each year, then the probability of fixation of the INA^- genotype for a y year period is $1-(1-p)^y$. We calculate the probability of fixation with release rates of 10^{-2}, 10^{-3}, and 10^{-4}. For the worst case, the probability of fixation could be very high (0.39) if INA^- bacteria are used for 50 years (Table 10). The longer the INA^- bacteria are used, the higher the probability that they will eliminate the INA bacteria (Table 10). The probability of fixation, however, is extremely sensitive to the release rate. At more realistic rates of release, the neutral gene model predicts that elimination of INA bacteria is much less likely, even for long periods of time (Table 10).

Finally, we calculate the time it would take INA^- bacteria to eliminate INA bacteria. Complete elimination of INA bacteria can only occur in theoretical models with finite population sizes. Expected fixation times have been calculated for these models (Crow and Kimura, 1970) but the calculations would be complex and involve several *ad hoc* assumptions in our case. Thus we use the far simpler model with infinite population size that was developed by Wright (1949). Instead of calculating the time to fixation, we calculate the expected half-time to the equilibrium gene frequencies calculated above. For selectively neutral alleles this time is $t = -\ln(0.5)/ u_i$. We calculate half-times for a variety of release rates when INA^- bacteria are released in only one year (Table 10). The half times vary from a relatively fast 69 years for our worst case and 690 - 6300 years for more realistic scenarios. The half times involved for the more realistic cases imply that genetic shifts would occur slowly.

These results can be generalized for when INA bacteria have a selective advantage, as is hypothesized in Chapter 8. In particular, if INA bacteria have some selective advantage over INA^- bacteria, then the equilibrium gene frequency of INA^- alleles would be lower, the fixation probabilities of INA^- alleles would be lower, and the half-time to equilibrium would be longer than in the neutral case. In other words, the selectively neutral case is the worse case.

There is one major problem with this global analysis. Global spatial averaging reduces the probability that two competing organisms can coexist (Okubo, 1980, p. 200, ff.). Thus more local analysis is necessary.

We relax the restrictive assumption that INA^- and INA^+ genotypes are ecologically identical and selectively neutral. Instead, we assume that their interaction is symmetrical, that is, INA^- bacteria affect INA^+ bacteria the same way that INA^+ bacteria affect INA^- bacteria. Similarly, INA^- bacteria affect themselves the same way INA^+ bacteria affect other INA^+ bacteria. What is different is that INA^- (or INA^+) bacteria might affect themselves differently than how they affect the other. Indeed there are two possibilities: first that INA$^-$ (or INA$^+$) bacteria inhibit themselves more than the other, and second that they inhibit themselves less than the other. The first case leads to stable coexistance in all circumstances. It simply describes the condition that intra-strain competition is greater than inter-strain competition. The second case leads to elimination of one strain within a patch. Which is the winner depends on which establishes large enough populations earliest. In this case, inter-strain competition is greater than intra-strain competition.

Practically, either of these situations might prevail. Nothing is known. Theoretically, the first case might be more likely. If bacteria occur in small single-strain colonies on the leaf surface, and competition is local to the colony, then competition within strains is likely to be greater than competition between strains.

In a heterogeneous environment, if intra-strain competition were greater than inter-strain competition and dispersal were small enough, no release of INA$^-$ bacteria, no matter how large, would have a lasting global effect on INA populations (Levin, 1976). How small dispersal must be is not known for the general case. In general, both strains will tend to the stable 2-strain equilibrium within each patch, no matter how many there were to start (Levin 1978). Long term impacts would not occur. This does not, however, imply that transient global or local effects would not occur.

If the second case prevails, then global theory would predict that one type will eliminate the other, depending on the initial conditions. For our problem, INA$^-$ bacteria would be constantly added, so in our global analysis, it would displace all INA bacteria.

In a spatially heterogeneous environment, both types could coexist if dispersal were small enough (Gopalsamy, 1977; Levin, 1974; 1978; Okubo, 1980; Shigesada, et al., 1979). For the two patch case Levin (1974) showed that the two strain equillibrium is locally stable if $0 \leq D \leq (r/2)(b-a)/(2b+a)$, where D is an exchange rate proportionality constant, r is the intrinsic rate of increase, and b and a are the respective inter- and intra-strain competition coefficients. D varies from between 3.6×10^{-3} to 4.0×10^{-6} (Chapter 7), r is about 0.05 (median value, Chapter 6), $a = r/K$, $K = 10^{8}$, so $a = 5 \times 10^{-10}$, and b can only be guessed at. However $b > a$. Assuming b is on the same order of magnitude as a, possibly $b = 10 \times 10^{-10}$, then $(r/2)(b-a)/(2b+a) = 5 \times 10^{-3}$, and the local stability criterion is likely to hold. These figures are calculated for spatial scales near 10m.

If this result could be generalized to the n-patch case (this has not been proven, but Yodzis, 1978 presents a strong argument that it can be generalized), then we would conclude that locally stable states occur where either INA^- or INA bacteria predominate. The global stability of these local states is not known (Levin, 1976; 1978), but they can persist for long periods of time (Yodzis, 1978). In other words, both INA^- and INA bacteria would coexist, but locally one or the other would dominate. Thus, persistent local effects of INA^- bacteria could occur.

Both arguments generalize for asymmetrical cases where one of the strains is a slightly better competitor than the other (Levin, 1978). Clearly, the qualitative form of competition between INA^- and INA bacteria is critical for predicting the impact of widespread releases of INA^- bacteria.

Local Scale

The theoretical analysis suggests that at spatial scales of 10 meters, dispersal is low enough that both INA and INA$^-$ bacteria could successfully coexist, if neither had been given an overwhelming advantage.

Before accepting this result, we model spread of INA$^-$ bacteria. INA$^-$ bacteria have several unique properties in their population dynamics (see Chapters 5, 6, and 7). These properties include: 1) actively growing bacteria disperse at high rates, 2) the variance in r, the intrinsic rate of increase, is extremely large from day to day, and 3) competition appears to occur by site limitation.

We simulate invasion and spread of INA$^-$ bacteria using several modifications of the model in Appendix 6. The model is a diffusion-reaction model (Levin, 1974) and is similar to the simulation model of Kampmeijer and Zadoks (1977). Spread and growth occurs in a randomly varying homogeneous environment. Variation is uncorrelated in space and time.

We find that the particular details of the biology of INA/INA$^-$ bacteria do not affect the qualitative pattern of spread. Invading INA$^-$ bacteria spread out uniformly from a release point, rapidly reach a constant rate of spread (by time = 10), and increase population density rapidly. The rate of spread depends primarily on D (the movement constant) and r (the intrinsic rate of increase). These results can be demonstrated theoretically (see Okubo 1980).

Spread is affected quantitatively by the biological details of INA/INA$^-$ bacteria. When dispersal is proportional to the local rate of population increase, spread and population growth are much slower. When the variance in r is greater, spread and population growth are faster.

Finally, when invasion occurs against a competitor, spread and population growth are slower. When dispersal is proportional to local population increase and the variance in r is high, spread and population growth are faster. If competition also occurs, spread is slightly slower and population growth is much slower.

In general, our simulations show that the more realistic details of INA/INA$^-$ population dynamics do not greatly affect our theoretical results. In particular, at the local spatial scale of 10 to 100 meters, dispersal is low enough that stable coexistence of INA and INA$^-$ bacteria should be possible, if both are allowed to establish. Coexistence would occur by a spatial division of the environment. Areas where INA or INA$^-$ bacteria were abundant should both be found. This means that: 1) If large areas were covered with releases of INA$^-$ bacteria early enough, then INA bacteria could be eliminated from the local environment. 2) If INA$^-$ bacteria are applied to only part of the environment, then the applied INA$^-$ bacteria and the wild bacteria should coexist, with INA$^-$ bacteria dominating the agricultural fields and wild bacteria dominating the natural areas.

Meso- and Continental Scales

At these larger spatial scales, dispersal is less than at the local scale. This implies that it becomes more difficult for INA$^-$ bacteria to successfully displace INA bacteria. Levin's (1974; 1976; 1978) results indicate that competitive coexistence occurs in heterogeneous environments when dispersal is low enough. Since dispersal is less at larger spatial scales, it should be easier for INA and INA$^-$ bacteria to coexist. Hence it should be more difficult for INA$^-$ bacteria to displace INA bacteria at these larger spatial scales than at the local scale.

Summary

The spread of widescale releases of INA^{-} bacteria is on several spatial scales: local, meso-, continental, and global. We analyse the spread potential of INA^{-} bacteria at these spatial scales.

For our global argument we assume a homogeneous environment with complete mixing of INA^{+} and INA^{-} genotypes. We reach the following conclusions:

1) In our worst case analysis, where a maximum amount of agricultural land receives INA^{-} bacteria for 50 years, the probability that INA^{-} bacteria completely displace INA bacteria is high, 0.39. The rate of replacement could be high, with a half-time to equilibrium of 69 years.

2) Under more realistic conditions (c.f. Chapters 4 and 11), the probability that INA^{-} bacteria displace all the INA bacteria is much smaller, 0.005 to 0.05. The rate of replacement is extremely slow, with a half-time to equilibrium of 690 to 6000 years. A process that takes this long would probably not be a dominant force in nature.

We relax our assumptions, and assume a heterogeneous environment with incomplete mixing of INA^{+} and INA^{-} genotypes. These assumptions lead to a spatially structured model that is more realistic than our first model. The main conclusions are:

1) At the local scale (10 - 100 meters) we can parameterize the model with results from the empirical

literature. We conclude that the dispersal rate is low enough that spatial heterogeneity can result in competitive coexistence of INA and INA$^-$ bacteria. In other words, there are conditions at the local scale under which INA$^-$ bacteria could not displace all the INA bacteria.

2) There are, however, conditions at the local scale under which INA$^-$ bacteria could displace all the INA bacteria. If INA$^-$ bacteria are released early enough, and in great enough quantity, they could eliminate all the INA bacteria. This is not a particularly surprising result, but the theoretical considerations imply that this is the only condition that INA$^-$ bacteria could displace all INA bacteria. This result is much more restrictive than the result for uniform, well mixed environments.

3) At larger spatial scales (meso- and continental), dispersal is less than at the local scale. This implies that competitive coexistence is more likely at these scales than at the local scale, and that it is much less likely that INA$^-$ bacteria would displace all the INA bacteria.

4) The conditions under which INA$^-$ bacteria could displace INA bacteria at the larger spatial scales are unlikely. For this to happen, huge areas (100,000 km^2) would have to be inundated with INA$^-$ bacteria early in the growing

season. This does not seem feasible. Such a uniform, widespread application of other agricultural chemicals does not now occur.

In conclusion, the probability that INA$^-$ bacteria displace a major portion of the INA bacteria in large spatial domains is highly unlikely, perhaps much less than 0.005, and the rate that this would occur, if it were to occur is extremely slow, taking 1000's of years. Thus, longterm, permanent, global impacts of INA$^-$ bacteria are extremely unlikely. Short term, local, or transient impacts, however might be more likely. Displacement of INA bacteria at local scales could occur if enough INA$^-$ bacteria are released early enough. Close attention should be paid to local effects.

Chapter 10: INA Bacteria and Precipitation

While there is ample evidence that the concentration of ice nuclei in the atmosphere can limit precipitation locally, it is not known how much ice nuclei limit precipitation overall. Two types of nuclei are important for the development of precipitation: cloud condensation nuclei and ice nuclei. Ice nuclei do not cause precipitation in nonfreezing clouds, such as predominate in the tropics. However, for freezing clouds in tropical montane and nontropical regions, ice nuclei may determine rain formation, since ice nuclei are scarce in the atmosphere (Rogers, 1979). Weather modification by cloud seeding is based on the premise that ice nuclei are a limiting factor for precipitation. While the evidence is still equivocable, recent analysis demonstrates that cloud seeding frequently enhances rain production (Bigg, in press).

The relative importance of plant epiphytic INA bacteria as atmospheric ice nuclei is highly debatable (Rogers, 1979; Schnell et al., 1981). The issue can be addressed as two different questions. First, what is the relative importance of warm temperature ice nuclei in overall precipitation patterns, and second, what is the relative contribution of leaf derived INA bacteria to the total concentration of warm temperature ice nuclei in the atmosphere. The conclusions that we reach are that warm temperature ice nuclei can be important in some clouds in some conditions, and that globally, the contribution of leaf derived ice nuclei is probably small. Leaf derived ice nuclei, however, may be quantitatively important locally. The major uncertainty is whether INA bacteria in leaf litter and decaying vegetation are the same as those on leaf surfaces.

Importance to Precipitation

The importance of INA bacteria to precipitation is related to the temperature of ice nucleation in clouds. If ice nucleation in clouds typically occurs at warm temperatures greater than -8°C, then INA bacteria will probably be more important. A thorough discussion of the temperature of ice nucleation for different cloud types, however, is beyond the scope of this analysis. Instead we present evidence that in some cases INA bacteria might influence precipitation.

Direct evidence to this point is unavailable. INA bacteria occur in the atmosphere (Flanagen and Jayaweera, 1980) and actively nucleate ice at temperatures approaching zero in cloud chamber testing (Table 1, chapter 3). Ice crystals occur in clouds with temperatures near -5° (Rogers, 1979; Schnell, 1979); and almost 50% of the ice nucleating particles collected from thunderstorms by Rosinski et al. (1980) were biologically derived and about 1 to 10 m in radius. Thus, bacteria are strongly implicated.

The relative importance of warm temperature ice nuclei, however, can be disputed. Rogers (1979) argued that the majority of ice nucleation in clouds might occur near -15° C. Many particles nucleate ice formation at this temperature. Still, ice is found in clouds at temperatures approaching 0°C, such as when a cloud is ascending into freezing air, and the nuclei responsible for ice crystal formation in these circumstances deserve further study (Rogers, 1979; Maki, 1974; Schnell et al., 1981).

Cumulus clouds typically contain many more ice nuclei than would be expected from the relatively warm temperature in the cloud and the concentration of particles in the cloud that are active ice nuclei at that temperature (Mossop et al., 1970; 1972). Mason (1973) argued that these extra nuclei appear because the air in a cumulus cloud moves vertically for great distances. When this happens, the temperature in the cloud can drop as much as 8°C, thus allowing many particles to form ice crystals. This process diminishes the importance of warm temperature ice nuclei in these clouds. Even so, warm temperature nuclei may play a role in stratocumulus clouds, which do not show this behavior (Mason, 1973), or in some tropical clouds (Snider et al., unpublished manuscript).

We conclude that in some conditions, the concentration of INA bacteria, can limit precipitation. We could not determine how frequently this occurs.

Concentration of INA Bacteria

If INA bacteria are important in cloud ice formation, then INA bacteria should be found in clouds in sufficient numbers to be effective ice nucleators. Information about atmospheric INA bacteria is virtually nonexistent, so we shall discuss general findings on bacterial concentrations in the atmosphere.

Atmospheric bacterial concentrations have rarely been monitored, and in only a few cases have efforts been made to measure the ice nucleating ability of bacteria from atmospheric sampling. A range of $40/m^3$ to $1.7 \times 10^3/m^3$ of INA bacteria has been found near plant canopies by two different studies (Schnell et al., 1981; Lindemann et al., 1979). In Wyoming, from air samples containing total bacterial concentrations of about $50/m^3$ to $500/m^3$, Maki found two isolates with INA activity, both of which later were found to be *P. syringae* (Maki, personal communication). Over the Arctic Ocean, Flanagen and Jayaweera (1980) found INA bacteria in an average bacteria concentration of 1.5 x $10^5/m^3$ of atmosphere. Atmospheric bacteria concentrations vary from none detectable to over 10^6 per m^3 (Akers et al., 1979; Bovallius et al., 1980; Flanagen and Jayaweera, 1980; Gregory, 1973; Maki, personal communication). The higher observed concentrations of INA bacteria are sufficient to account for observed concentrations of ice nuclei in some clouds, but the lower values are not. This is an area demanding further research. Improved estimation of the potential for INA bacteria to contribute to the formation of cloud ice crystals is essential.

Role of Leaf Derived INA Bacteria

Atmospheric INA bacteria can originate from several sources. Three major sources have been identified: leaf surfaces, decaying organic matter (Schnell and Vali, 1976), and ocean upwellings (Schnell and Vali, 1976). The relative importance of these sources to total atmospheric INA bacteria concentrations is currently unknown. Each source, however, may dominate locally.

Oceanic sources were first identified by Schnell and Vali (1976) in the Antarctic Ocean. They correlated atmospheric concentrations of INA bacteria with the occurence of oceanic upwellings, and identified an algal species, *Heterocapsa niei*, as having ice nucleating activity. Later, Parker et al. (unpublished manuscript) found that *P. flourescens*, which was associated with the algae, actually accounted for the oceanic ice nucleating activity. Upwellings provide nutrients for algal and bacterial population increases, and may provide the turbulance to create waves strong enough to launch bacterial aerosols. Oceanic sources elevate atmospheric INA bacterial concentrations locally; they probably have little impact on concentrations far inland.

The relative importance of decaying organic matter and leaf surfaces as sources of terrestrially-derived INA bacteria is unknown. When terrestrially-derived biogenic ice nuclei were first identified, Schnell and Vali (1976) implicated decaying organic matter. Since then, however, they have been less certain, suggesting both organic matter sources (Schnell, 1979), and leaf tissue sources (Schnell et al., 1980). This is an important unresolved issue.

Leaf epiphytic INA bacteria are primarily P. syringae and E. herbicola, as discussed above, but it is not yet known what INA bacteria species dominates decaying organic matter. Both P. flourescens and P. syringae have been found (Maki et al., 1975; unpublished data). P. flourescens is usually thought to dominate many soil habitats, but because much decaying organic matter is leaf-derived, high innocula of P. syringae might also be expected. Without this information, even an approximate estimate of the impact of widespread releases of INA bacteria is severely impaired.

Several assumptions can be made. In the worst case, decaying organic matter would contribute no INA bacteria to the atmosphere, and our analysis reduces to that in Chapter 9. Another possibility is that all of the INA bacteria in decaying organic matter would be P. syringae. The analysis in Chapter 9 would still hold, however, the quantitative result would change. Since more naturally occurring INA bacteria would exist, invasion and displacement of INA bacteria would be less likely, and the probability of significant environmental impact would be less. In the best case, the INA bacteria in decaying organic matter would be P. flourescens, and this species would be the major source of atmospheric ice nuclei. Then, even if INA$^-$ P. syringae displaced all INA P. syringae, there would be relatively little environmental impact. Depending on these assumptions, a slight, but real environmental risk, or no environmental risk of widespread use of INA$^-$ bacteria would be predicted.

Local Effects

While the average concentration of atmospheric INA bacteria is not known, it is probably much less than the average concentration of ice nuclei in a cloud. Thus, local variability in atmospheric concentration and in the size of local sources of INA bacteria will probably have greater effects on precipitation patterns than globally average concentrations. These local effects have not been conclusively documented, but correlative evidence and theoretical models indicate that they are possible.

Bigg (in press) proposed a model to explain long term changes seen in precipitation patterns after cloud seeding experiments. The model involves secondary ice nuclei, whereby rainfall was enhanced by secondary, and more effective, ice nuclei after cloud seeding attempts using silver iodide. He suggests that these secondary ice nuclei were produced from plants, following exposure to silver iodide. Plants developed spots after treatment with silver iodide and produced increased quantities of ice nuclei. The derived ice nuclei were not identified, but some may be INA bacteria.

Similarly, Bryson (1973) suggests that reduction in plant derived ice nuclei might contribute to desertification. He hypothesized that a drought in the Sahel was related to a decrease in plant populations, and thus a decrease in plant-derived ice nuclei in the atmosphere to provide nucleation sites for rain. This model has been further supported and expanded by work of Schnell (1975) and Sands et al. (1982; 1985). They suggest, in addition, that the reduced rainfall, coupled with intensified grazing pressure on the vegetation, further reduced the amount of vegetation and accelerated desertification.

Summary

Current understanding of the role of INA bacteria in determining precipitation patterns is poor, so it is impossible to assess quantitatively the likely impact of widespread releases of INA^- bacteria. Qualitatively, the greatest possible impact would be region-wide desertification. One must assume, however, 1) that leaf epiphytic INA bacteria are the most important source of atmospheric INA bacteria, 2) that *P. syringae* is rare in decaying organic matter, 3) that atmospheric INA bacteria are the major warm temperature ice nuclei, 4) that precipitation is limited by the concentration of warm temperature ice nuclei, and 5) that precipitation limits production of terrestrial INA bacteria. While some of these conditions are likely to hold in some regions, they might not occur concurrently. Thus, the overall risk of desertification from widespread release of INA^- bacteria is likely to be very small. This likelihood must be determined on a local level, taking into account the geographic particularities of the region.

Chapter 11: Modelling INA- Bacteria on Potato

In chapter 4, we estimated the total frost sensitive cropland in the world to be about 7.1 x 10^8 ha. In our subsequent analysis we assumed that INA^- bacteria would be applied to all of this area. This probably overestimates the use of INA^- bacteria in agriculture, because INA^- bacteria will be applied to crops only when it is economically feasible. While economic feasibility can be greatly affected by political policy, we discuss the use of INA^- bacteria on potatoes under contemporary United States economics conditions.

Potatoes make a good model crop to evaluate the potential for use of INA^- bacteria in agriculture. Potatoes are a major world food crop (F.A.O. Production Yearbook, 1984), and are grown in most food production regions of the world (Swaminathan and Sawyer, 1982). INA^- bacteria will be tested on potatoes (Lindow, 1985b), and may be used in commercial production. Potato yields might be limited by frost at the end of the growing season before the crop has reached maturity (Burton, 1981). If frost damage were decreased then potato tubers could continue to fill for a longer time period.

Potato is a frost sensitive crop (Li et al., 1981). In the vast majority of widely grown potato cultivars, frost injury invaribly occurs at temperatures colder than -2^0C in the absence of supercooling (Li et al., 1981; Hellmers and Warrington, 1982). Potatoes are generally resistant to chilling injury from exposure to temperatures above zero, and even from subzero temperatures if frost formation is prevented (Lindstrom and Carter, 1985). Thus potato cultivars generally have very poor frost tolerance and relatively high levels of chilling injury resistance (Li et al., 1981; Lindstrom and Carter, 1985). If ice nucleation is prevented, potatoes could survive a frost and resume growth at warmer temperatures. Average growth of commercial potato tubers per day is about 400 kilograms per hectare (Burton, 1981). However, chilling damage is cumulative and, after a relatively long period of cold temperatures (four days at -4^{0}C) permanent damage will be done to potato shoot tissues (Lindstorm and Carter, 1985).

Prevention of frost damage to potatoes is complicated by temperature dependent freezing damage in potato. Supercooling followed by ice formation can increase plant injury dramatically compared to ice initiation at warmer temperatures (Rajashekar et al., 1983; Burke et al., 1976). Deliberate addition of ice nuclei to leaves of moderately frost resistant plants might cause supercooling to temperatures where freezing would be extremely injurious (Rajashekar et al., 1983).

Yield Loss Assessment

Potato yield loss from early frosts is estimated by computer simulations using the potato growth model "SpudGrow" (Johnson, et al., 1986). This potato yield simulator has been verified with field data from Minnesota and performs well (Johnson et al., 1986; P.S. Teng, unpublished data). Inputs to the model are soil water status, minimum and maximum daily temperatures, planting date, and tuber bulking date. We compiled a frequency distribution of first frosts in several potato production regions, including Minnesota/North Dakota, Idaho, Maine, and northern California. For each region, we calculated 30 year averages of published temperature data, and assumed that there would be sufficient water in the soil for potato growth (many areas are irrigated). Limitations to this analysis are discussed in Appendix 7.

The distributions of first fall and last spring frosts in the four regions are shown in Figure 4. The growing season from median last spring frost to median first fall frost was: Pocatello, ID, 115 days; Mt. Shasta, CA, 132 days; Fargo, ND, 132 days; Bangor, ME 154 days. The range in the time of last spring frost was about 60 days at Pocatello, ID and Mt. Shasta, CA and about 30 days at Fargo, ND and Bangor, ME. The range in the time of first fall frost was about 50 days at Mt. Shasta, CA, 45 days at Bangor, ME, 37 days at Pocatello, ID, and 25 days at Fargo, ND.

The thirty year mean daily maximum and minimum temperatures and the average of the maximum and minimum temperatures are also shown in Figure A7-2. Diurnal temperature fluctuations were greatest for Mt. Shasta, CA and Pocatello, ID, and least for Fargo, ND, and Bangor, ME. The averages of the maxima and minima were about the same in all regions.

Potato growth was simulated for three emergence dates at all regions (50 percent emergence on May 20, June 1, and June 15) for cultivar Russet Burbank. The age of the plants in physiologic age units is shown in Figure 5. Simulations of growth were stopped on September 20.

We calculated potential yield loss from frost as follows: we assumed that fall frost resulted in complete destruction of the haulm, so no yield increase was possible after frost; we determined the effect of complete defoliation at various physiologic ages by simulated defoliations (Figure A7-1, also in Johnson et al., 1986).

Physiologic maturity occurs at 800 physiologic age units for Russet Burbank. If the 50 percent emergence date occurred after the last spring frost, potatoes would easily reach physiologic maturity before the first fall frost at Fargo, ND and Bangor, ME. Since both Wisconsin and New York potato growing areas experience conditions milder than these, use of INA$^-$ bacteria in any of these production areas is unlikely.

At Mt. Shasta, CA and Pocatello, ID yield loss from frost can occur (Figure 5). The probability of a certain yield loss is different at the two sites and for different planting dates. With earlier planting dates, the tail of the distribution rises and the probability of very high yield loss increases. This occurs because the last spring frost damages more mature plants as planting date is earlier. Late planting at Pocatello is more risky than late planting at Mt. Shasta (June 15), but early planting at Mt. Shasta has a greater risk of very high yield loss than at Pocatello.

At both sites there is at least a 0.1 probability of at least 10 percent yield loss for any planting date. In other words, in at least one year out of 10, frost will result in at least a $90.00 to $124.00/acre yield loss in these regions. At Mt. Shasta, CA, the situation is a little worse; in at least one year in 12, frost will result in at least $180.00 to $248.00/acre yield loss. At Pocatello, ID, yield loss is strongly dependent on planting date.

Assuming that expected loss must be greater than control costs we calculate the maximum expected loss for each region by planting date combination (Figure 6). Maximum expected yield loss from frost at Pocatello, ID, depended strongly on planting date, varying from just under $10.00/acre to about $55.00/acre. Maximum expected yield from frost at Mt. Shasta, CA was less variable and always greater than about $15.00/acre.

If the total cost of use of INA$^-$ bacteria were less than $10.00 to

\$15.00/acre, and use were 100 percent effective at stopping frost losses, then use might be economically feasible. Typical insecticide application costs are about \$5.00 to \$10.00 per acre, so perhaps only one and occasionally 2 applications of INA$^-$ bacteria could be economically feasible. INA$^-$ bacteria might be as expensive as other microbial pesticides or expensive pyrethroids, and would cost \$10.00 to \$20.00/acre. If both spring and fall frosts threaten potato yield, as they do for some planting dates at both sites, then at least two applications would be required, and use of INA$^-$ bacteria would be economically feasible only for late planted potatoes at Pocatello, ID. If either the spring or fall application were forgone, then the treatment would not be 100 percent effective at protecting the plants from frost (Appendix 7). Frost occurring during the unprotected season could reduce yield. Finally, the efficacy of INA$^-$ bacteria in field conditions is unknown, but it is likely to be somewhat less than 100 percent, as is commonly observed in other pesticides.

We conclude that use of INA$^-$ bacteria may be economically feasible on potatoes in some rather restricted conditions. In the United States only northern California and Idaho potato growers stand to benefit. Within this group, only growers with late planted potatoes in Idaho are likely to see this as profitable, and only if INA$^-$ bacteria are reasonably effective. Perhaps less than 5 percent of the potatoes grown in the United States satisfy these criteria.

If use of INA$^-$ bacteria is economically feasible, then only a few potato growing regions would benefit, viz. probably northern California and

Idaho. This could enhance the economic advantage of these two regions compared to other potato producing areas in the country. Northern California primarily supplies a local market. Thus greater efficiency in production should decrease potato prices. Idaho, on the other hand, ships a large proportion of its potatoes to other regions. The greater efficiency in production of Idaho potatoes could alter the balance in regional competition for the potato market (W.B. Sundquist, personal communication). This would be a complex analysis, but these effects should be investigated.

Summary

We examine potato in greater depth and argue that while potato is a frost vulnerable crop, there is little potato acreage in the United States where application of INA$^-$ bacteria would be economically feasible. Our analysis is based on a comparison of maximum potential gain and reasonable estimated costs. We calculate maximum potential gains by simulating yield loss from frost induced defoliation with a computer model and estimating probability distribution functions for yield loss from historical frost records. We calculate expected losses from these two data and equate them with maximum potential gains. Reasonable values of cost are determined from analogous pesticides. We conclude that perhaps less than 5 percent of United States potato acreage will ever receive applications of INA$^-$ bacteria. Actual use of INA$^-$ bacteria is likely to be far less than we speculated in chapter 4.

Chapter 12: Synthesis

To start our analysis we develop an absolute worst case scenario (chapter 2). This scenerio demonstrates that there is a possibility for wide-spread application of modified INA$^-$ bacteria to significantly affect climate. The quantity of INA$^-$ bacteria available to move into the atmosphere could be three orders of magnitude greater than the quantity of INA bacteria in the atmosphere. INA$^-$ bacteria could replace natural INA populations. This result could be cause for concern, but we believe that circumstances will not fulfill the assumptions of this absolute worst case scenario. Instead, we conclude that the possibility of climatic impact cannot be summarily dismissed as out of hand, and that a *biologically constrained worst case scenario* should be developed.

Prior to developing the biologically constrained worst case scenario, we evaluate the ability of various particles to nucleate ice formation (chapter 3). All particles can nucleate ice formation if it is cold enough. At temperatures warmer than -5°C, however, there are very few sources of ice nuclei. The main types are living bacteria, dead bacteria, some inorganic particles, some crystaline chemicals, and "intrinsic" plant structures. Living INA bacteria are certainly important sources, and crystaline chemicals are certainly not. Inorganic particles are believed to be unimportant, but this has not received as much attention. The role of dead bacteria and structures that are intrinsic to plants is still disputed.

The most common and active INA bacterial species are probably *Pseudomonas syringae, P. fluorescens, Erwinia herbicola* and *P. viridiflava*.

Of these, P. syringae is particularly notable because of its high abundance and the high frequency of INA isolates. There are, however, probably many more INA species. Other species should not be dismissed for their sparseness. There may be some potent INA species that play important roles in plant freezing. We will, however, focus our analysis on P. syringae.

INA bacteria are found in both marine and terrestrial habitats. Global analysis of the impact of widespread releases of INA^- bacteria should consider both sources. Local analysis could restrict attention to terrestrial sources, which are living and decaying vegetation. To develop a biologically constrained worst case scenario, we assume that INA bacteria are the major source of ice nuclei above -5°C.

The structure of our biologically constrained scenario follows the process of invasion and spread in new habitats. First, we determine the cropland area that INA^- bacteria could be applied. Second, we examine the interactions of INA and INA^- bacteria on the phylloplane to estimate bacterial population density and the rate of displacement of INA bacteria by INA^- bacteria. Third, we estimate dispersal of phylloplane bacteria within habitats and between habitats and model the potential spread of INA^- bacteria. Finally, we consider the impact of reduced INA bacterial populations on precipitation patterns.

Cropland

Our estimate of cropland on which INA^- bacteria might be applied includes about 1.1 x 10^8 ha in the United States and 7.1 x 10^8 ha in the world (chapter 4). This is approximately 50 percent of the world's cultivated land and 10 percent of the world's land surface (F.A.O. Production Yearbook, 1985). We estimate a total leaf area of frost vulnerable cropland to be about 3.3 x 10^8 ha in the United States and 2.1 x 10^9 ha in the world. These are clearly overestimates. Some cropland that rarely experiences frost damage is included. In addition, we do not consider problems of efficacy and use of INA^- bacteria. Instead, we assume that INA^- bacteria are effective, that they are relatively inexpensive, and that they are readily available. Finally, we include both wheat and corn as host sensitive crops. These two crops account for half of our estimated land area.

Our assumption that nearly 10 percent of the world's surface could have INA^- bacteria applied is probably high (chapter 4). This, however, should be the absolute maximum. If we eliminate wheat, corn, a few low value crops, and some tropical crops from consideration, a more reasonable maximum acreage is 1 percent of the world's surface. Finally, since even highly advantageous technologies are not perfectly used, it is unlikely that even 1 percent of the world's surface will receive INA^- bacteria. Thus, more realistic maximum values of total cropland area are from 0.1 to 1.0 percent of the world's surface. This does, however, assume that INA^- bacteria will be economically feasible.

We examine the economic feasibility of INA^- for use on potato in the United States (chapter 11). We argue that while potato is a frost vulnerable crop, there is little potato acreage where application of INA^- bacteria would be economically feasible. Our analysis is based on a comparison of maximum potential gain and reasonable estimated costs. We calculate maximum potential gains by simulating yield loss from frost induced defoliation with a computer model and estimating probability distribution functions for yield loss from historical frost records. We calculate expected losses from these data and equate them with maximum potential gains. Reasonable values of cost are determined from analogous pesticides. We conclude that perhaps less than 5 percent of United States potato acreage will ever receive applications of INA^- bacteria. If this result generalizes, then between 0.005 to 0.05 percent of the world's surface might receive INA^- bacteria.

Interactions

The natural history of leaf epiphytes is poorly understood (chapter 5). Little is known about the biotic interactions of leaf epiphytes. For example, predators and parasites are assumed to be virtually non-existant. Despite this, many grazing arthropods, such as mites, collembola, and thrips, must consume many bacteria. Without basic information on natural history, many critical assumptions must be made about how leaf epiphitic bacteria do or do not interact.

We make the following assumptions about bacterial interactions on the phylloplane (chapters 5 and 6): (1) host plant and physical factors dramatically affect epiphytic bacteria; (2) predators, parasites, and mutualists have little effect on ephphytic bacteria; (3) competition by ephphytic bacteria is a scramble competition for resources, either nutrients or sites; (4) both INA and INA^- bacteria can successfully overwinter in the habitats they can grow.

For scramble competition for resources, a resource depletion model with a carrying capacity (maximum sustainable population density) is appropriate (chapter 6). The carrying capacity of a unit of habitat fluctuates wildly in time and is highly variable in space. Thus, even in habitats with high mean population densities, there is some non-zero probability of successful colonization, because there are under utilized sites. Typical INA densities range from not detectable to $10^{7.4}$/gfw, with an interquartile range of $10^{2.6}$ to $10^{5.0}$/gfw. Total bacteria density has an interquartile range of $10^{4.8}$ to $10^{6.8}$/gfw. Using our calculated variance to mean ratio we infer that in 25 percent of the potential P. syringae environments, at least 0.24 or 0.0067 percent of the leaf sites have fewer than 100 INA bacteria or total bacteria respectively. These sites are likely candidates for colonization.

The growth rate of P. syringae is highly variable with time, but there are long term trends. Daily intrinsic growth rates usually vary from -1.61 to 1.61 day^{-1}, with a maximum of 5.04 day^{-1}. Longer term growth rates (22 - 100 days) vary from 0.013 to 0.082 day^{-1} (median = 0.048 day^{-1}).

Ice nucleation activity might provide INA bacteria a competitive advantage in certain situations (chapter 8), by allowing increased population growth by conditioning the resource, or by increasing the efficiency of atmospheric dispersal. Released INA^- bacteria would not have either of these advantages; this would decrease the ability of INA^- bacteria organism to spread and establish. Most of the evidence, however, indicates no selective difference between the INA^- and INA^+ genotype. We assume that INA^+ and INA^- genotypes are selectively neutral, which enhances the probability that INA^- genotypes will spread.

Although we assume that INA^+ and INA^- genotypes are selectively neutral we do not assume that they are identical (chapter 9). Instead, we assume that competition is symmetrical. If interstrain competition were less than intrastrain competition, then INA^- bacteria could not displace INA bacteria. If interstrain competition were greater than intrastrain competition, then INA^- bacteria could displace INA bacteria. For our scenario, we assume that interstrain competition is greater than intrastrain competition.

Dispersal and Spread

Movement of P. syringae on the phylloplane might occur on a scale of 1 cm. This implies that bacteria on a leaf might not interact if they are far enough apart, and that our concept of a "site" ($50\ cm^2$) is much too large. We may be grossly underestimating the occurence of under-exploited sites in the field. We use, however, the larger "site" in our analysis.

Dispersal agents of P. syringae are not well characterized (chapter 7). Wind and rain splash are important agents, and similar to other bacteria, anthropogenic activities and insects may be important agents. We focus on the first three.

The movement of P. syringae from leaf surfaces into the atmosphere could occur during mechanical operations in agriculture and during rainless days. Potato haulm pulverizing can project 2.2×10^8 cfu/ha of bacteria into the atmosphere, and hay combining can project 2.3×10^{12} cfu/machine - hour. In neutral to unstable atmospheric conditions on rainless days, about 7.0×10^9 cfu/ha/hour can be projected. The greater the epiphytic density, and the larger the agricultural field, the greater was the movement of P. syringae into the atmosphere during rainless days. Rain redistributed bacteria in a field, added colonists, reduced local population density, and probably created conditions favorable for bacterial growth.

Focusing on wind transport, we examine transport on five spatial scales: micro, local, meso, continental, and global. The mathematical models most appropriate for these scales might be: micro -- passive diffusion; local -- turbulent diffusion/Gaussian Plume Model; meso -- patches of habitat with uniform dispersal among patches; continental -- patches of habitat of randomly varying quality connected by a stochastic dispersal process; global -- no transport model is needed. Long distance dispersal probably occurs at significant non-zero rates.

Deposition of bacteria from the atmosphere to the leaf is much higher during rain (740-4200 cfu/50 cm^2/hour) than during dry periods (15-47 cfu/50cm^2/hour). Bacterial survival is likely to be higher during wet deposition than during dry deposition.

We analyze spread potential of INA$^-$ bacteria in two steps. First, we assume that INA$^-$ bacteria invade a homogeneous environment with complete mixing of INA$^+$ and INA$^-$ genotypes. Second, we relax this assumption and discuss spread in a heterogeneous environment and spatially structured populations of INA$^+$ and INA$^-$ genotypes. The first approach yields quantitative estimates of invasion potential, while the second is more realistic.

In a homogeneous environment, where a maximum amount of agricultural land receives INA$^-$ bacteria for 50 years, the probability that INA$^-$ bacteria completely displace INA bacteria is high, 0.39. The rate of replacement could be high, with a half-time to equillibrium of 69 years.

Under more realistic conditions when less agricultural land receives INA$^-$ bacteria, the probability that INA$^-$ bacteria displace all the

INA bacteria is much smaller, 0.005 to 0.05. The rate of replacement is extremely slow, with a half-time to equillibrium of 690 to 6000 years. A process that takes this long would probably not be a dominant force in nature.

In a heterogeneous environment, our conclusions depend on spatial scale. At the local scale (10 - 100 meters) we parameterize a model with results from the empirical literature. We conclude that the dispersal rate is low enough that spatial heterogeneity can result in competitive coexistence of INA and INA$^-$ bacteria. In other words, there are conditions at the local scale under which INA$^-$ bacteria could not displace all the INA bacteria.

There are, however, conditions at the local scale under which INA$^-$ bacteria could displace all the INA bacteria. If INA$^-$ bacteria were released early enough and in great enough quantity, they could eliminate all the INA bacteria. This is not a particularly surprising result, but the theoretical considerations imply that this is the only condition that INA$^-$ bacteria could displace INA bacteria. This result is much more restrictive than the analogous result for homogeneous environments.

At larger spatial scales (meso- and continental), dispersal rates are less than at the local scale. This implies that competitive coexistence is more likely at these scales than at the local scale, and that it is much less likely that INA$^-$ bacteria would displace all the INA bacteria.

The conditions under which INA$^-$ bacteria could displace INA bacteria at the larger spatial scales are unlikely. For displacement to occur, huge areas (100,000 km^2) would have to be innundated with INA$^-$

bacteria early in the growing season. This does not seem feasible. Such a uniform, widespread application of other agricultural chemicals does not now occur.

The probability that INA^- bacteria displace a major portion of the INA bacteria in large spatial domains is less than 0.005, and the rate that this would occur, (if it were to occur) is extremely slow, taking 1000's of years. Thus, long term, permanent, global impacts of INA^- bacteria are extremely unlikely. Short term, local, or transient impacts, however, might be more likely. Displacement of INA bacteria at local scales could occur if enough INA^- bacteria are released early enough. Attention should be paid to potential local effects.

Precipitation

Current understanding of the role of INA bacteria in determining precipitation patterns is poor, so it is impossible to quantitatively assess the likely impact of widespread releases of INA^- bacteria. Qualitatively, the greatest possible impact would be region-wide desertification. One must assume, however, 1) that leaf epiphytic INA bacteria are the most important source of atmospheric INA bacteria, 2) that *P. syringae* is rare in decaying organic matter, 3) that atmospheric INA bacteria are the major warm temperature ice nuclei, 4) that precipitation is limited by the concentration of warm temperature ice nuclei, and 5) that precipitation limits production of terrestrial INA bacteria. While some of these conditions are likely to hold in some regions, they might not all hold simultaneously. Thus, the overall risk of desertification from widespread release of INA^- bacteria is likely to be very small. This likelihood must be determined on a local level, taking into account the geographic particularities of the region.

Conclusions

1. Widespread release of INA$^-$ bacteria in agriculture is not likely to cause long-term or global effects on precipitation.

Release rates are not likely to be very high. Possible rates, including subsequent reproduction, are 10^{-2} to 10^{-5} of the total bacterial population. Probable rates are nearer 10^{-5}. These probable rates derive from 1) not all of the cropland planted to frost sensitive crops is frost vulnerable, 2) it will not be profitable to use INA$^-$ bacteria on all frost vulnerable cropland, and 3) INA$^-$ bacteria will not be used in all cases even if it is more profitable.

In the probable case, spread will occur very slowly, taking thousands of years and the probability that INA bacteria would be eliminated is very small, less than 0.005.

In the worst case, spread could occur quickly, taking about 100 years, and the probability that INA bacteria would be eliminated is very high, 0.39. The most critical assumption is that inter-strain competition is greater than intra-strain competition. If this were not true, then the probability that INA bacteria would be eliminated by INA$^-$ bacteria is near zero.

2. Widespread release of INA$^-$ bacteria in agriculture could cause significant local or short-term effects on precipitation.

Local displacement of INA bacteria can occur if enough INA$^-$ bacteria are released early enough in the growing season. Theory predicts that these populations could exclude large populations of INA bacateria.

The critical assumptions are many: 1) Bacteria are the only important ice nucleating agent at warm (-5°C to 0°C) temperatures; 2) P. syringae is the important bacterial species; 3) INA^- and INA^+ genotypes are selectively equivalent; 4) Inter-strain competition is greater than intra-strain competition; 5) Leaf epiphytic INA bacteria are the most important source of atmospheric INA bacteria; 6) P. syringae is rare in decaying organic matter; 7) Atmospheric INA bacteria are the major warm temperature ice nuclei; 8) Precipitation is limited by the concentration of warm temperature ice nuclei.

If all these assumptions bold, then the worst effect could be local desertification. High value, frost sensitive crops are frequently grown in large areas surrounding metropolitan areas. A shift in precipitation in these areas could have a major impact on many people. Since many of our assumptions can be easily tested at minimal cost, we believe that they should be tested immediately.

3. Predictions of the potential ecological impact of INA^- bacteria, or of any other biologically engineered microorganism, are based on information about the natural history of these organisms. For microorganisms, and specifically for INA bacteria, this information is severely lacking. In particular, details of competition and predation, dispersal, adaptive value, and ecosystem-level functioning are lacking. We believe that research should be initiated in the basic ecology of microorganisms.

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Figure Legends

Figure 1 (for Chapter 2). Steps in the spread of INA$^-$ bacteria and in the displacement of INA bacteria.

Figure 2 (for Chapter 6). Probability that a site will have fewer than 100 bacteria as a function of density, based on corn data in Hirano et al., 1982. Actual values for soybean and oats are higher than predicted from the corn data.

Figure 3 (for Chapter 6). Median and interquartile ranges of average INA and total bacteria population density on various plants. Data from Lindow, Arny and Upper, 1978.

Figure 4 (for Chapter 10). Distributions of last spring and first fall frosts, and physiologic development of potato cultivar Russet Burbank for three 50 percent emergence dates at Mt. Shasta, CA; Fargo, ND; Pocatello, ID; and Bangor, ME. See text and appendix 7 for details.

Figure 5 (for Chapter 10). (a) Cumulative probability distribution functions of potato yield loss from frost for cultivar Russet Burbank at Mt. Shasta, CA and Pocatello, ID for three 50 percent emergence dates. (b) Expected potato yield loss from frost for cultivar Russet Burbank at Mt. Shasta, CA and Pocatello, ID for three 50 percent emergence dates.

Figure 6 (for Chapter 10). Maximum expected potato yield loss from frost for cultivar Russet Burbank at Mt. Shasta, CA and Pocatello, ID for three 50 percent emergence dates.

Fig 1

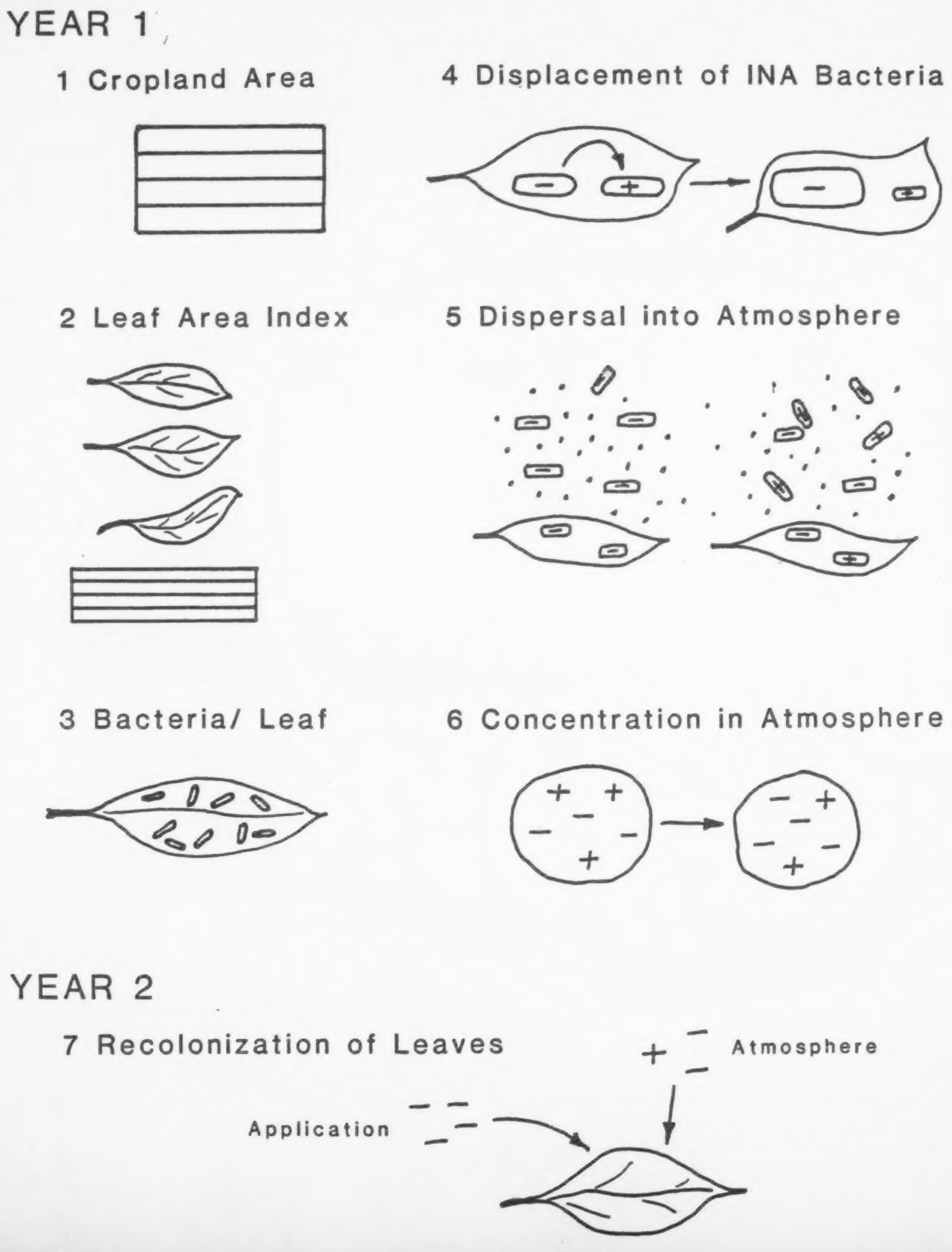
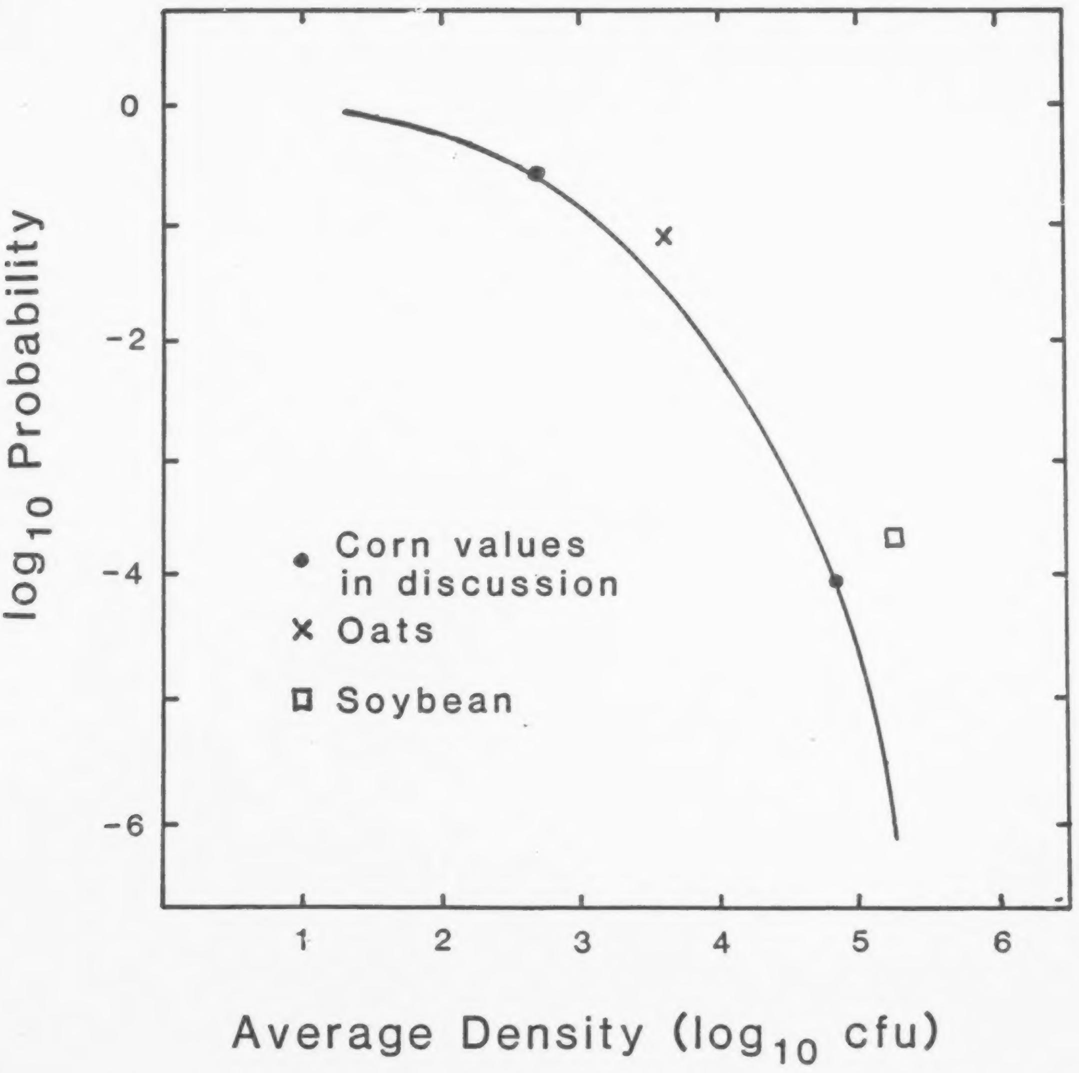
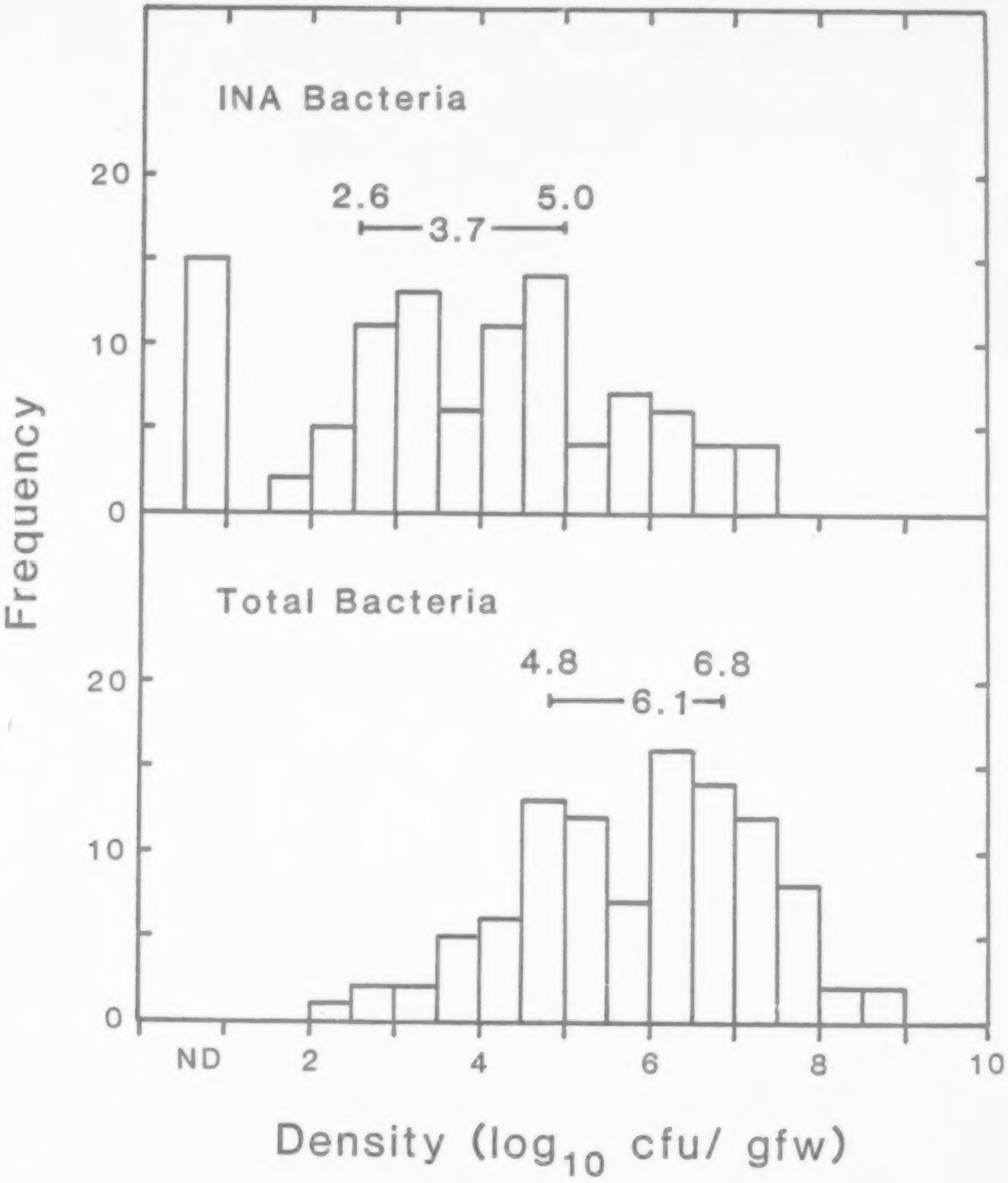

YEAR 1
1 Cropland Area
2 Leaf Area Index
3 Bacteria/ Leaf
4 Displacement of INA Bacteria
5 Dispersal into Atmosphere
6 Concentration in Atmosphere
YEAR 2
7 Recolonization of Leaves
Atmosphere
Application

Fig 2



0
-2
-4
-6
1
2
3
4
5
6
log₁₀ Probability
Average Density (log₁₀ cfu)
● Corn values in discussion
× Oats
□ Soybean

Fig 3


INA Bacteria
20
2.6
3.7
5.0
10
0
Frequency
Total Bacteria
20
4.8
6.1
6.8
10
0
ND
2
4
6
8
10
Density (log10 cfu/ gfw)

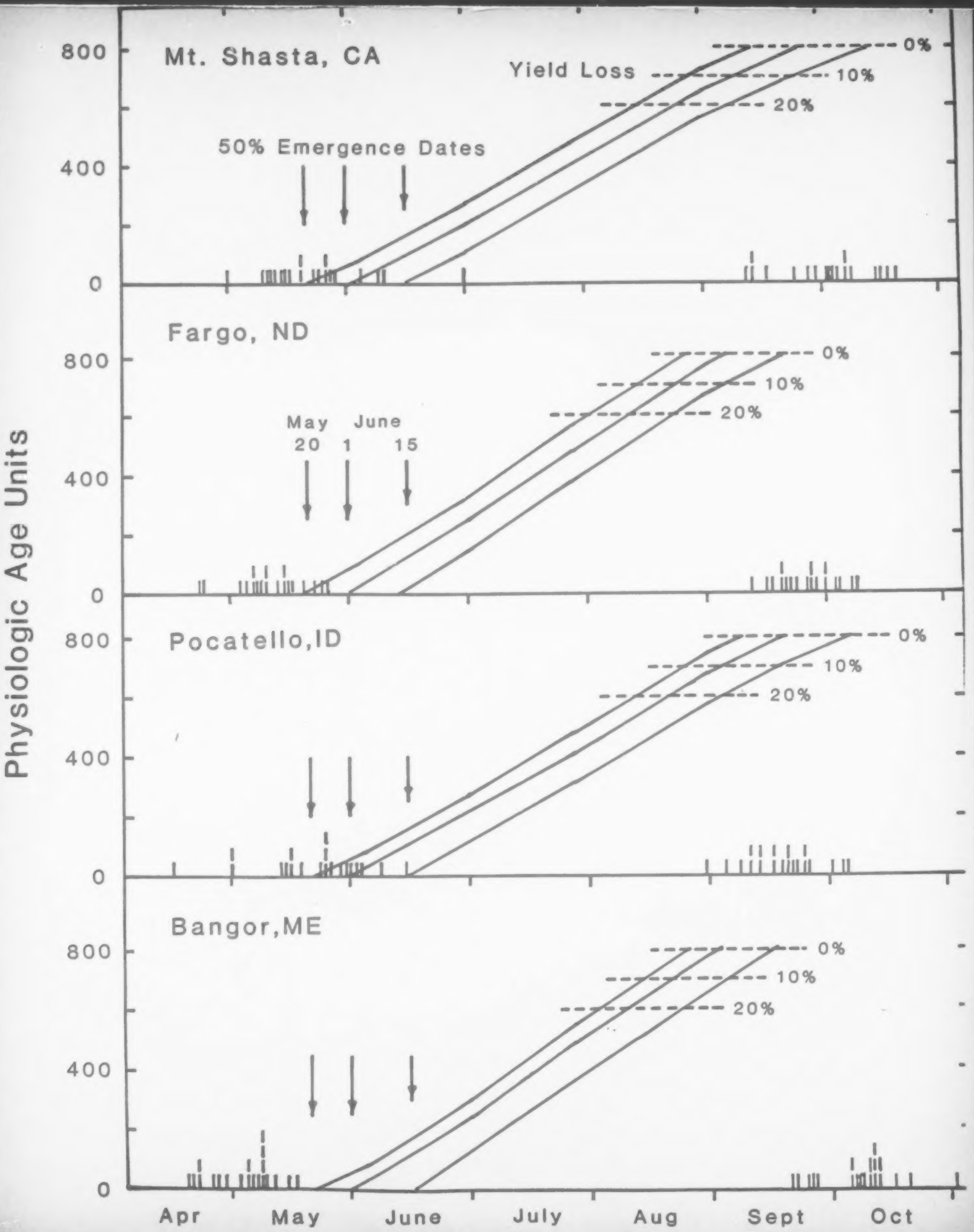

Physiologic Age Units
Mt. Shasta, CA
Yield Loss
50% Emergence Dates
Fargo, ND
May 20
June 1
15
Pocatello, ID
Bangor, ME
0%
10%
20%
0
400
800
Apr
May
June
July
Aug
Sept
Oct

Fig 5

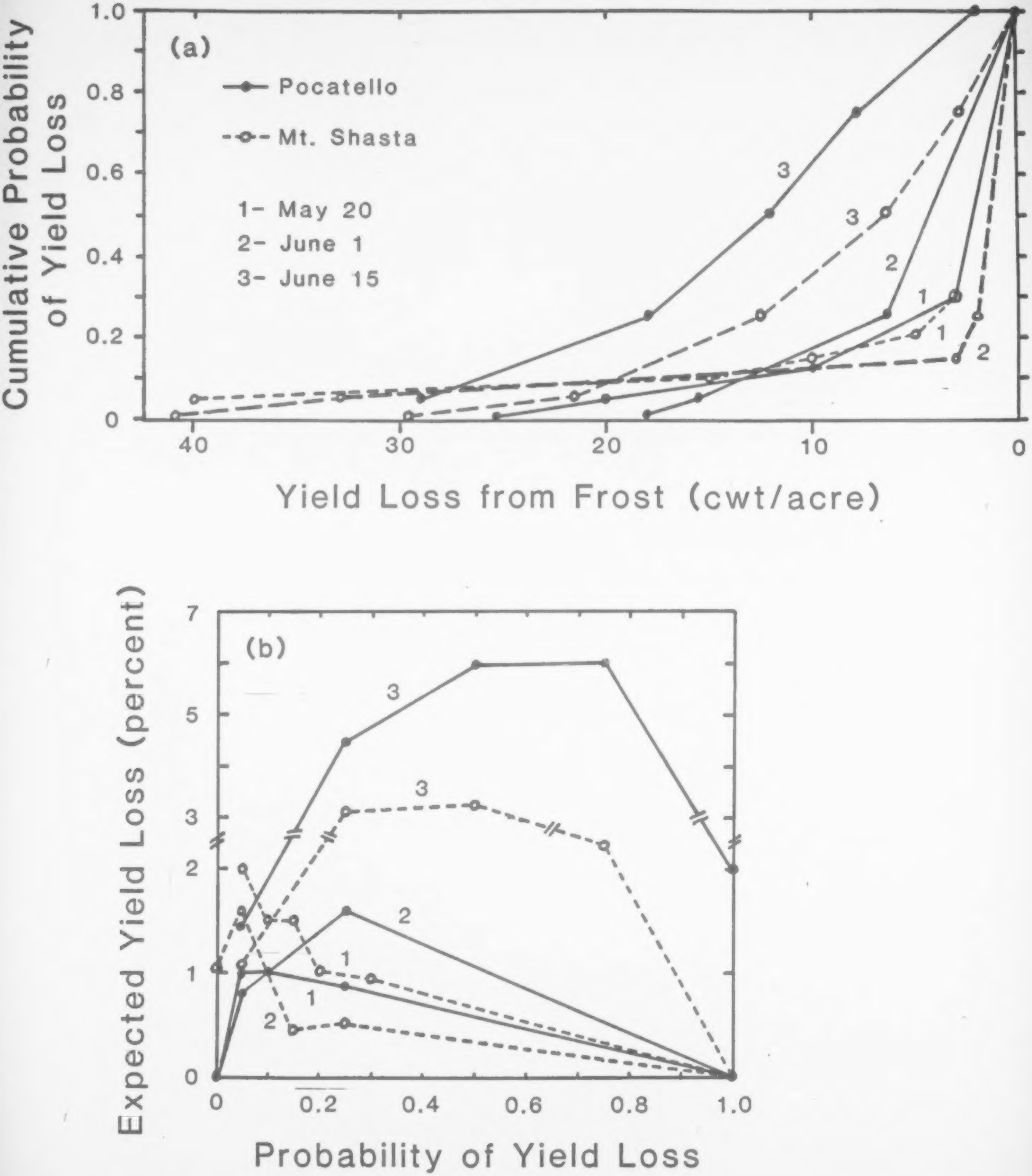
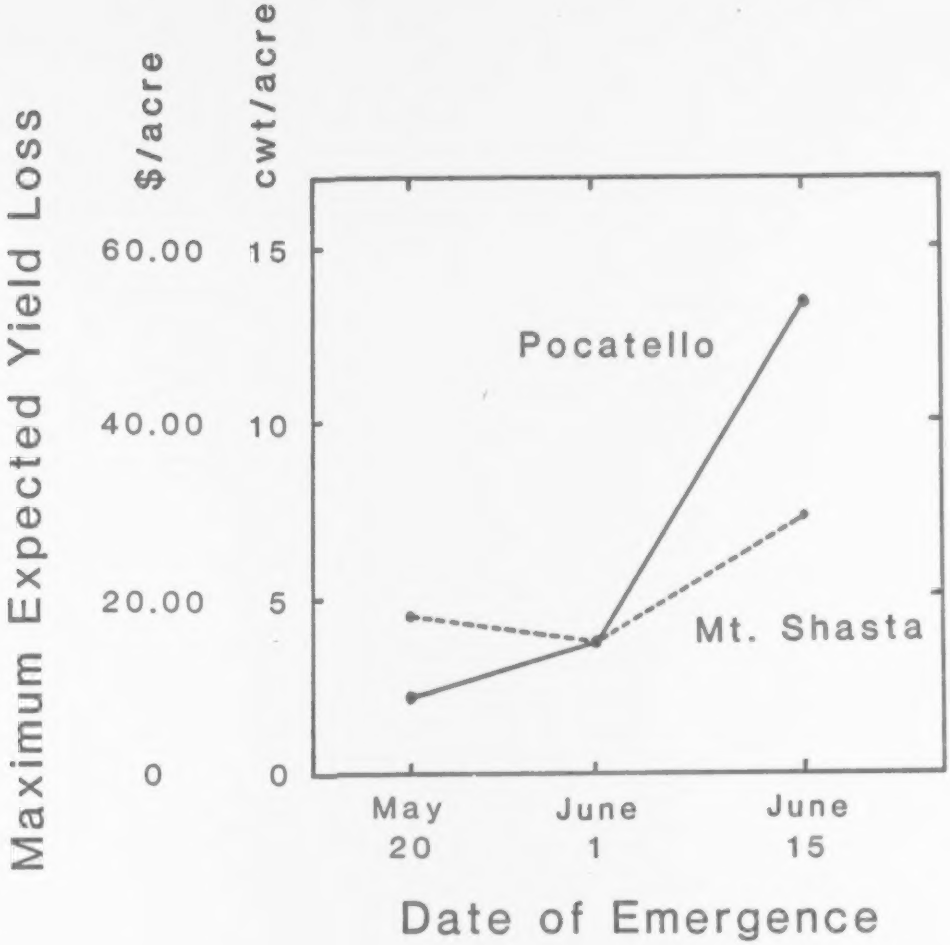

(a)
Pocatello
Mt. Shasta
1- May 20
2- June 1
3- June 15
Cumulative Probability of Yield Loss
1.0
0.8
0.6
0.4
0.2
0
40
30
20
10
0
Yield Loss from Frost (cwt/acre)
1
2
3
(b)
Expected Yield Loss (percent)
7
5
3
2
1
0
0
0.2
0.4
0.6
0.8
1.0
Probability of Yield Loss
1
2
3

Fig 6


Maximum Expected Yield Loss
$/acre
cwt/acre
60.00
40.00
20.00
0
15
10
5
0
Pocatello
Mt. Shasta
May 20
June 1
June 15
Date of Emergence

Appendix 1: Bacteria Population Density

Estimates of maximum INA^- Bacteria Populations on Plant Surfaces

Reference	Spp (Crop/Bacteria)	Range[1]
Crosse, 1959	Cherry leaves/*P. s. syringae*	Undetectable to 1.54×10^5 cells/leaf
Ercolani et al., 1974	Hairy vetch leaves/ *P. s. syringae*	Maximum>10^7 cells/gfw Range: $10^4 - 10^7$
Gross et al., 1984	Apple buds/ *P. s. syringae*	10 to 10^6 cfu/gfw average = 5×10^3 cfu/gfw
Hirano et al., 1985	Oat leaves/INA spp.	$10^2 - 10^7$ cfu/leaf Lognormal dist: Mean = $10^{3.6}$, V = $10^{1.18}$
Hirano and Upper, 1985b	Snap beans leaflet/INA spp.	Maximum = 10^8 cfu/leaflet
Lindow et al., 1978c	Leaves, Soybeans, Beans, Tomato, Pumpkins/INA spp.	Undetectable (about 10^2) to 10^8 (cells/gfw)
Lindow et al., 1982b	Leaves, Tomato/INA spp. (Citrus, Avocado:Low Pop.)	Maximum = $10^{5.5}$ cells/gfw
Lindow et al., 1978a	95 plant species tested for INA, 74 positive (Conifers had low INA)	Total bacteria pop.: $10^3 - 10^8$ INA pop. = 0.01 to 7% of total bacteria pop.

[1] gfw = gram fresh weight,

cfu = colony forming units

detection threshold is about 100 cfu,

oat leaf is about 30 cm^2, and a bean leaflet is about 50 cm^2

Troposhere volume

Earth's radius (6.38 x 106m) + average ht of the troposphere (15,000m) = 6.395 x 106 m. The volume of a sphere is 4/3 pi r^3. Thus, the troposphere volume is

$$4/3\ (6.395 \times 10^6\ m)^3\ pi - 4/3\ (6.38 \times 10^6 m)^3\ pi = 7.7 \times 10^{18}\ m^3.$$

Appendix 2: Simple Spread Model

Consider the following two states: atmosphere and phylloplane.

Let $N_A^+(t)$ = Number of INA bacteria in atmosphere at time t,

$N_A^-(t)$ = Number of INA$^-$ bacteria in atmosphere at time t.

Observe that N_A (t+1) = number of bacteria in atmosphere at time t, minus the number that die, minus the number that wash out and colonize phylloplane surface, plus the number of bacteria reaching the atmosphere from the phylloplane. For this analysis we can ignore reproduction by bacteria that were not atmospherically derived. Including them in the analysis does not change the qualitative conclusions. Thus, we drop the subscripts. In general, each of the relationships described above, i.e., mortality, washout, reproduction, and dispersal, are complicated relationships. In this analysis, however, we assume that the IN$^-$ and IN bacteria are in all of these ways similar, so without loss of generality we can consider only linear functions. (Mathematically any non-linear relation can be expanded into a Taylor series. All linear terms can be collected and separated from the higher order terms. In this analysis, the behavior of each of the higher order terms is identical to the behavior of the linear terms.) Thus,

$$N^+(t+1) = N^+(t) - k_1N^+(t) - k_4N^+(t) + k_3k_2k_1N^+(t), \text{ and}$$

$$N^-(t+1) = N^-(t) - k_5N^-(t) - k_8N^-(t) + k_7k_6k_5N^-(t) + N_c,$$

where

k_1, k_5 = washout rates to natural plants

k_4, k_8 = mortality rates,

k_2, k_6 = epiphytic reproduction rates on natural plants

k_3, k_7 = atmospheric loading rates from natural plants, and

Appendix 2: Simple Spread Model (continued).

N_c = atmospheric loading rate from crop plants.

These equations simplify to:

$N^+(t+1) = N^+(t)\ (1-k_1-k_4+k_3k_2k_1)$, and

$N^-(t+1) = N^-(t)\ (1-k_5-k_8+k_7k_6k_5) + N_c$.

Consider the quantity $P = N^+/N^-$. Observe that if N^+ becomes relatively rarer than N^- then P decreases. Examine $P(t+1) - P(t)$, to determine if N^+ becomes rare compared with N^-.

$$P(t+1) - P(t) = \frac{N^+(t+1)}{N^-(t+1)} - \frac{N^+(t)}{N^-(t)} = \frac{N^+(t+1)}{N^-(t+1)} \quad \frac{N^+(t)}{N^+(t)} - \frac{N^-(t+1)}{N^-(t)} .$$

Since $N^+(t)/N^-(t+1) > 0$, the sign of $P(t+1) - P(t)$ is determined by the parenthetical term. Substituting in the dynamic equations for $N^+(t+1)$ and $N^-(t+1)$, we obtain:

$$(1-k_1-k_4+k_3k_2k_1) - (1-k_5-k_8+k_7k_6k_5) - N_c/N^-(t).$$

The assumptions that IN and IN$^-$ bacteria have similar biologies implies that $k_1 = k_5, k_4 = k_8, k_2 = k_6$, and $k_3 = k_7$. Therefore the sign of

$P(t+1) - P(t)$ is the same as $-N_c/N^-(t) < 0$,

and we conclude that with agricultural loading, INA bacteria will become relatively rarer than INA- bacteria in the atmosphere, and the higher the agricultural loading, the faster this will happen. A more general argument for these conclusions is developed in chapter 9.

Appendix 3. Determination of Ice Nucleation Activity

Droplet Freezing Technique (Vali, 1971).

Aqueous suspensions with a concentration of 1-6g solid matter/100ml dist.H2O of the material to be tested (in this case, fresh or decaying plant leaves) were prepared. Thirty 10 microliter droplets are placed on a temperature controlled surface and the temperature is slowly lowered from ambient to -25C. The temperatures required to freeze 10, 50, and 90% of the droplets are recorded. An ice nucleation spectrum is produced which enumerates the number or concentration of ice nuclei active at each temperature step. The assumptions are that time-dependence is not a factor and that each nucleus has a specific freezing temperature. These assumptions were not explicitly tested here.

Aluminum Foil Boat Technique (Lindow, 1982b).

This is an adaption of droplet freezing technique. Bacterial cells are suspended in water or 0.1M phosphate buffer. Bacteria grown in broth were diluted. Viable cell counts were determined by dilution plating. Ice nucleation concentrations at a range of temperatures were determined by placing droplets on parafin coated aluminum foil boats floating on cooled ethanol/water mixture. The temperature is lowered and the ice nucleation spectrum is determined (Vali, 1971).

Test Tube Freezing (Paulin and Luisetti, 1978).

Tubes with a suspension (10^9 cells/ml) of the bacteria to be tested are put into a salt water bath cooled to -4C for 5 minutes. The isolate was INA if the tube froze.

Appendix 3: Determination of Ice Nucleation Activity (continued).

Leaf Disk Technique (Lindow, 1978a)

In a modification of the Foil Boat Droplet freezing technique, corn leaf disks (3mm diam.) were immersed in a droplet of water and cooled. The temperature at which the drop froze was recorded.

Tube Nucleation Test (Hirano et al., 1985).

Leaves are removed from plants and placed into a test tube with a buffer solution. The tubes are submerged in successively colder baths, from -2.5 to -4.0, and the temperature at which each tube freezes is recorded. About 95% of ice nucleation events occurred during the first 30 min of a 4-5 hour test. Comparison of gradual versus sudden cooling of plant tissue showed no difference.

Replica Plate Technique (Lindow ,1978a).

Plants in Florida, Wisconsin and California were sampled. The leaves were washed in buffer solution and washings plated out onto agar media. Replicas of colonies were transferred to parafin coated aluminum foil by means of a velvet pad. The foil was cooled to -5C and misted with water. Patches of ice found where colonies of INA+ bacteria were present.

Micropipette Technique (Makino, 1982).

The micropipette technique was compared with the droplet freezing technique. Micropipettes (10 microliter) were filled with the material to be tested. They were cooled in an ethanol/water bath. Concentrations of 10^5 cells/microliter ($=10^8$ cells/ml) were used. The standard error of the micropipette technique was smaller than that of the droplet freezing technique.

Appendix 3: Determination of Ice Nucleation Activity (continued).

Membrane Filter (Schnell et al., 1981).

Schnell describes a membrane filter technique which measures atmospheric deposition ice nucleation. A membrane which has been exposed to the material to be tested is placed in a dynamic thermal diffusion chamber. The chamber is supplied with water vapor and cooled. The appearance of ice crystals is visually determined.

Appendix 4. INA bacteria species.

Species	Number of Tested Isolates	Number of INA Isolates	Authority
Pseudomonas syringae	134	106	Paulin and Luisetti, 1978
Pseudomonas viridiflava	19	8	Paulin and Luisetti, 1978
P. group 1 (morsprunorum, phaseolicola, savastanoi, tomato)	39	4	Paulin and Luisetti, 1978
Pseudomonas group 3,4,5	19	0	Paulin and Luisetti, 1978
non-fluorescent Pseudomonads	51	0	Paulin and Luisetti, 1978
Erwinia amylovora	24	0	Paulin and Luisetti, 1978
Erwinia carotovora	8		Paulin and Luisetti, 1978
Erwinia herbicola var ananas	1	1	Paulin and Luisetti, 1978
Erwinia stewartii	1	0	Paulin and Luisetti, 1978
Erwinia herbicola	52	0	Paulin and Luisetti, 1978
Xanthomonas spp. (juglandis, maculifoliigardenniae, vitians)	23	0	Paulin and Luisetti, 1978
Corynebacterium spp (fascians, flaccumfaciens, michiganense)	4	0	Paulin and Luisetti, 1978
Agrobacterium tumifaciens	3	0	Paulin and Luisetti, 1978
Bacillus spp.	9	0	
Pseudomonas syringae	2[1]	2	Maki et al., 1974
P. aeruginosa	1	0	Maki et al., 1974
Staphylococcus epidermidis	1	0	Maki et al., 1974
Escherichia coli	1	0	Maki et al., 1974
Enterobacter aerogenes	1	0	Maki et al., 1974
Proteus mirabilis	1	0	Maki et al., 1974
Proteus vulgaris	1	0	Maki et al., 1974
Bacillus subtilis	1	0	Maki et al., 1974
Bacillus cereus	1	0	Maki et al., 1974
Various isolates	271	37[2]	Maki and Willoughby, 1978
Pseudomonas syringae	8	3	Makino, 1982
P. andropogonis	1	0	Makino, 1982
P. avenae	1	0	Makino, 1982
P. cissicola	1	0	Makino, 1982
P. fluorescens	1	0	Makino, 1982
P. marginalis	1	0	Makino, 1982
Erwinia carotovora	2	0	Makino, 1982
Erwinia herbicola	3	0	Makino, 1982
Erwinia milletiae	2	0	Makino, 1982
Xanthomonas campestris	13	0	Makino, 1982
Corynebacterium michiganense	1	0	Makino, 1982
C. flaccumfaciens	1	0	Makino, 1982
Pseudomonas syringae[6]	105	65	Herano et al., 1978
Pseudomonas syringae	50[3]	1	Vali et al., 1976
Many isolates	?	?[4]	Maki and Garvey
Pseudomonas syringae[6]	67	65	Lindow et al., 1978a
Pseudomonas syringae[6]	109	98	Lindow et al., 1982[5]

Appendix 4. INA bacteria species (continued)

Species	Number of Tested Isolates	Number of INA Isolates	Authority
P. syringae-like	28	28	Lindow et al., 1982[5]
P. fluorescens	3	1	Lindow et al., 1982[5]
P. marginalis	1	0	Lindow et al., 1982[5]
P. aeruginosa	1	0	Lindow et al., 1982[5]
P. solanacearum	1	0	Lindow et al., 1982[5]
Erwinia herbicola	6	2	Lindow et al., 1982[5]
E. herbicola-like	46	46	Lindow et al., 1982[5]
E. stewartii	4	0	Lindow et al., 1982[5]
E. carotovora	2	0	Lindow et al., 1982[5]
E. chrysanthemi	2	0	Lindow et al., 1982[5]
Escherichia coli	1	0	Lindow et al., 1982[5]
Xanthomonas campestris	2	0	Lindow et al., 1982[5]
X. axonapodia	1	0	Lindow et al., 1982[5]
Corynebacterium nebraskensis	4	0	Lindow et al., 1982[5]
Miscellaneous others	52	0	Lindow et al., 1982[5]
Erwinia stewartii	1	1	Wallin et al., 1979
Many isolates	?	?[7]	Gross et al., 1984
Pseudomonas syringae[7]	(82)	(82)	Gross et al., 1984
(8 - 11 species)	11	1[8]	Parker et al., m.s.
Pseudomonas syringae	105	51	Latorre and Jones, 1979
P. morsprunorum	96	23	Latorre and Jones, 1979

---

[1] This includes *P. syringae* C-9 from Vali et al., 1976.

[2] *Pseudomonas syringae*, *Pseudomonas fluorescens* biotype G.

[3] These are various uncharacterized bacterial isolates from decaying leaf litter (Schnell and Vali, 1976; LDN).

[4] Only *P. syringae* and *P. fluorescens* biotype G were active ice nuclei.

[5] Not clearly representative, may have selected for or against ice nucleating activity.

[6] May be some overlap, since all may have used Kelman isolates.

[7] Some colonies deviated from LOPAT, only INA isolates identified, only *P. syringae* found.

[8] White pigmented, sea-ice psychrophile; designated HK-31.

[9] Work by R.W. Fresh and S.A. Yankofsky is not included.

Appendix 5: Epiphytic Bacteria Species

The Concept of Bacterial Species

The taxonomic unit of bacteria is the clone or strain, an identical group of cells derived from one cell. Clones found in nature are grouped into subgroups for taxonomic study. Clones having sufficient characteristics in common are considered a species.

The taxonomy of bacteria is primarily based on morphological and metabolic features. G+C ratios have been recently incorporated into taxonomy.

Pseudomonaceae (Bergey, 1974)

The family Pseudomonaceae includes only gram negative, straight or slightly curved rods with polar flagella. They are aerobic and never fermentative. The family is diverse, including *Zooglea*, which forms large clumps in activated sewage sludge, *Gluconobacter*, which grows under acid conditions (pH 4.5) and oxidizes ethanol to acetic acid, *Xanthomonas*, a relatively fastidious plant pathogen and leaf epiphyte producing yellow pigments, and the type genus, *Pseudomonas*, a very diverse genus. Pseudomonads include plant pathogens, human and animal pathogens, soil inhabitants, mesophilic and psychrophilic species. Most can not grow below pH 6.0. The fluorescent species are distinguished by their production of diffusible yellow-green U-V fluorescent pigments. Some also produce blue orange or green phenazine pigments.

Pseudomonas fluorescens is often isolated from soil, and commonly is associated with spoilage of foods (eggs, milk, cured meats and fish), and occasionally with plant disease. It encompasses 5 biotypes, I,II,III IV and V (A, B, C, F and G of Stanier,1966). Biotype I is the typical

Appendix 5: Epiphytic Bacteria Species (continued).

Pseudomonas fluorescens, it is often isolated from soil and water, and associated with the spoilage of foods. Biotype II is occasionally phytopathogenic and includes species earlier named P. marginalis, and P. tolaasii. Biotype III and IV are distinguished by biochemical tests. Biotype V contains a heterogenous group of strains which cannot be assigned to any other group.

The genus Xanthomonas is similar to Pseudomonas, but most require amino acids for growth in vitro. Size ranges from 0.2-0.8 by 0.6-2.0 microns. Most species produce a yellow carotenoid diffusible pigment on agar media. They cause various plant diseases, on a wide range of plants. X. campestris causes vascular wilts in many Brassica spp.

Enterobacteriaceae (Bergey, 1974)

The family Enterobacteriaceae includes gram negative, motile or non-motile by peritrichous flagella, facultatitive anaerobes. They are oxidase negative and catalase positive, they produce acids from simple sugars. Many of them are human pathogens. The genus Erwinia is primarily composed of plant pathogens and plant epiphytes. The species possess a diverse assortment of metabolic substrates and products. They are all motile except for Erwinia stewartii. They are generally grouped into three groups: amylovora group, herbicola group and carotovora group. The amylovora group generally causes vascular wilts. The herbicola group contains an epiphyte, (Erwinia herbicola), a vascular wilt of maize, (Erwinia stewartii), and a species which attacks uredespores of Puccinia graminis. The carotovora group generally causes rots of storage and fleshy plant organs.

Appendix 5: Epiphytic Bacteria Species (continued).

Bacteria Species Found on Plants

Aerobacter
Acetinobacter
Azotobacter
Bacillus
Bacillus subtilus/licheniformis intermediates
Bacillus circulans
Bacterium
Beijerinckia
Chromobacterium
Erwinia herbicola
Erwinia amylovora
Erwinia carotovora
Erwinia herbicola var *ananas*
Erwinia stewartii
Flavobacterium aquatile
Flavobacterium pectinovarum
Klebsiella spp.
Kurthia spp.
Lactobacillus spp.
Listeria spp.
Micrococcus spp.
Mycobacterium
Pseudomonas aeromonas
Pseudomonas fluorescens
Pseudomonas marginalis
Pseudomonas melopthora
Pseudomonas putida
Pseudomonas syringae
Pseudomonas viridiflava
Sarcina
Staphylococcus spp.
Staphylococcus sapraphyticus
Spirillum
Xanthomonas
Xanthomonas campestris
Xanthomonas campestris pv. translucens

References

Blakeman, 1982
Crosse, 1971
Goodfellow et al., 1976
Lindow, 1985b

Appendix 6: Simple Spread Program with Modifications

```
Program Spread;
(**    Calculates the distribution and abundance of an invading      **)
(**    organism in a specified heterogeneous, patchy environment.   **)
(**    Population dynamics within a patch, emigration rates, and     **)
(**    a redistribution function must be specified.  Menus allow     **)
(**    choice among functional forms, and between deterministic      **)
(**    and stochastic elements.                                      **)


(*********************************************************************)
(*********************    Label Declarations    **********************)
(*********************************************************************)
label
  bottom,
  skip1,
  skip2,
  skip3,
  skip4;


(*********************************************************************)
(********************    Constant Definitions    *********************)
(*********************************************************************)
Const
  Dim1 = 21; (* Dimension 1, rows, of the environment grid *)
  Dim2 = 21; (* Dimension 2, collumns, of the envirnment grid *)
  D1 : Integer = Dim1;
  D2 : Integer = Dim2;
  MaxCellType = 20;


(*********************************************************************)
(****************    Definition of Variable Types    *****************)
(*********************************************************************)
Type
  CellType    = 1..MaxCellType;
  CellGrid    = array [1..Dim1,1..Dim2] of CellType;
  RealGrid    = array [1..Dim1,1..Dim2] of Real;
  CellParam   = array [1..MaxCellType] of Real;
  Word        = string[30];
  Arr1        = array [0..Dim1] of Real;
  Arr2        = array [0..Dim2] of Real;


(*********************************************************************)
(*******************    Variable Declarations    *********************)
(*********************************************************************)
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
Var
  EnvironmentGrid              : CellGrid;
  PopnGrid, DispGrid, MoveGrid : RealGrid;
  DistMat                      : RealGrid;
  PrtGrid                      : RealGrid;
  NoCellType                   : CellType;
  Time,i,j                     : Integer;
  r, rSD, K, KSD               : CellParam;
  A1,A2                        : Char;
  Grad1                        : Arr1;
  Grad2                        : Arr2;
  Name                         : Word;
  LeaveRate, Diffusivity       : Real;


(********************************************************************)
(*******************    Procedure Declarations    *******************)
(********************************************************************)

           (***********************************************)
           (********  Gets a named file from disk  ********)
           (***********************************************)
Overlay Procedure GetFile (var DiskFile: Text;var Name : Word);
Label
  label1;
begin
if Name = '' then
  begin
  label1: write ('Enter file name: ');               (*Read file name*)
  readln (Name);
  end; (*if*)
if Name = 'quit' then                               (*Quit if needed*)
  writeln('Quitting, could not find file.')
else
  begin
  assign (DiskFile, Name);
  {$I-}
  reset (DiskFile);
  {$I+}
  if IOresult <> 0 then                              (*Check name*)
    begin
    writeln('Cannot find ',Name,'.  Type "quit" if you want to quit.');
    goto label1;                                     (*Try again*)
    end; (*if*)
  end; (*else*)
end; (*of procedure GetFile*)

           (**************************************************)
           (********  Checks integer values in range  ********)
           (**************************************************)
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
Overlay Procedure  IntRange (var Out : Boolean; Value, Min, Max : Integer);
  begin
  Out := False;
  if (value < Min) or (value > Max) then
    begin
    Out := True;
    writeln('');
    writeln('Value out of range');
    end; (*if*)
  end; (*of procedure CellRange*)

           (****************************************************)
           (********  Checks cell type values in range  ********)
           (****************************************************)
Overlay Procedure CellRange (var Out: Boolean; Value, Min, Max: CellType);
  begin
  Out := False;
  if (value < Min) or (value > Max) then
    begin
    Out := True;
    writeln('');
    writeln('Cell type out of range');
    end; (*if*)
  end; (*of procedure CellRange*)

           (**************************************************)
           (********  Locates cells with given value  ********)
           (**************************************************)
Overlay Procedure Locate (Val:CellType; G:CellGrid; D1,D2:Integer; FNm:Word; var DFil:
var
  i,j     :Integer;
begin
assign (DFil, FNm);
rewrite (DFil);
for i := 1 to D1 do
  for j := 1 to D2 do
    if G[i,j] = Val then
      writeln (DFil, i,' ',j);
close (DFil);
end; (*of procedure Locate*)


           (*********************************)
           (********  Checks limits  ********)
           (*********************************)
Function Limit (High, X: Real): Real;
begin
if X <= 0.0 then
  Limit := 0.0
else
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
  begin
  if X >= High then
    Limit := High
  else Limit := X;
  end;
end;

           (*************************************************************)
           (********  Calculates normal R.V.s with given mean, SD  ********)
           (*************************************************************)
Function Normal (Mean, SD: Real): Real;
Function N01: Real; (*generates N(0,1) variates by Marsaglia-Bray "convenient"      *)
const               (*method, in Kennedy,WJ and Gentle,JE 1980 Statistical Computing*)
  c1 = 17.49731196;
  c2 = 4.73570326;
  c3 = 2.15787544;
  c4 = 2.36785163;
var
  x,y,g,U1  :Real;
begin
U1 := Random;
if U1 <= 0.8638 then
  N01 := 2*(Random + Random + Random - 1.5)
else
  if U1 <= 0.9745 then
    N01 := 1.5*(Random + Random -1)
  else
    if U1 <= 0.9973002039 then
      begin
      repeat
        x := 6*Random - 3;
        y := 0.358*Random;
        if abs(x) < 1 then
          g := c1*exp(-x*x/2) - c2*(3-x*x) - c3*(1.5-abs(x))
        else if abs(x) < 1.5 then
          g := c1*exp(-x*x/2) - c4*(3-abs(x))*(3-abs(x)) - c3*(1.5-abs(x))
        else if abs(x) < 3 then
          g := c1*exp(-x*x/2) - c4*(3-abs(x))*(3-abs(x))
        else g:= 0;
      until y < g;
      N01 := x;
      end
    else
      begin
      repeat
        repeat
          x := 2*Random - 1;
          y := 2*Random - 1;
          g := x*x + y*y;
        until g <= 1;
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
        if x > y then
          x := x*sqrt((9 - 2*ln(g))/g)
        else
          x := y*sqrt((9 - 2*ln(g))/g);
      until x > 3;
      N01 := x;
      end;
end;(*of function N01*)
begin
Normal := Mean + SD*N01;
end;(* of function Normal*)

           (********************************************)
           (********  Read Number of Cell Types  ********)
           (********************************************)
Overlay Procedure ReadNoCellType (var Num : CellType);
var
  TF    : Boolean;
begin
repeat
  write ('Enter the number of cell types (varieties, sites, etc): ');
  TF := False;
  {$I-}
  readln (Num);
  {$I+}
  if IOResult <> 0 then
    TF := True;
until TF = False;
end; (* of procedure ReadNoCellType *)

           (*****************************************************)
           (********  Initializes the environmental grid  ********)
           (*****************************************************)
Overlay Procedure InitEnv (var Num : CellType; var Grid : CellGrid; D1, D2 : Integer);
Label
  label1,
  label2;
Const
  Low       : Integer = 1;
Var
  Answer    : Char;
  GridFile  : Text;
  i,j,x,y   : Integer;
  Name      : Word;
  TF        : Boolean;
Procedure Change (var G : CellGrid; D1,D2:Integer; N:CellType);
var
  answer : char;
  x,y    : integer;
begin
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
   write ('Any changes? (Y/N)  ');
   readln (Answer);
   while (answer = 'Y') or (answer = 'y') do
    begin
    repeat
     write ('Specify grid coordinates (row,collumn):  ');
     readln (x,y);
     IntRange (TF, x, Low, D1);
     if TF = True then
      writeln('for row')
     else
      begin
      IntRange (TF, y, Low, D2);
      if TF = True then
       writeln('for column');
      end; (*else*)
    until TF = False;
    repeat
     write ('Enter new value:  ');
     readln (G[x,y]);
     CellRange (TF, G[x,y], Low, N);
    until TF = False;
    write ('Any more changes? (Y/N)   ');
    readln (Answer);
    end; (*while*)
end; (*of procedure Change*)
begin (*InitEnv*)
label2: clrscr;
writeln('Choose method to initialize the environment grid');
writeln('');
writeln('');
writeln(' a.  Random assignment of cell types to position');
writeln(' b.  Input from disk file');
writeln(' c.  Input from keyboard');
writeln('');
writeln(' q.  Quit');
writeln('');
label1: readln (answer);
case answer of
 'a': begin                    (***  random assignment of cell types  ***)
      for i := 1 to D1 do
      for j := 1 to D2 do
      Grid[i,j] := 1 + Random(Num);
      end; (*'a'*)
 'b': begin                              (***  from disk file  ***)
      Name := '';
      GetFile(Gridfile, Name);
      if Name = 'quit' then
       goto label2;
      reset (GridFile);
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
       for i := 1 to D1 do
         for j := 1 to D2 do
           begin
           read(GridFile, Grid[i,j]);

           CellRange (TF, Grid[i,j], Low, Num);
           if TF = True then
             writeln (i,' ',j,' ',Grid[i,j]);
           end; (*for*)
       close (GridFile);
       Change (Grid, D1, D2, Num);
       end; (*'b'*)
  'c': begin                                  (***  from keyboard  ***)
       clrscr;
       writeln ('Enter <cr> for each cell value');
       for i := 1 to D1 do
         begin
         writeln ('Enter the ',D2,' cell types for row ',i,':  ');
         for j := 1 to D2 do
           begin
           read (Grid[i,j]);
           CellRange (TF, Grid[i,j], Low, Num);
           if TF = True then
             writeln (i,' ',j,' ',Grid[i,j]);
           end; (*for*)
         writeln ('');
         end; (*for*)
       Change (Grid, D1, D2, Num);
       end; (*'c'*)
  'q': begin
       writeln ('Quitting...');
       end; (*'q'*)
  else begin                                   (***  mistake case 2  ***)
       writeln ('Try again');
       goto label1;
       end; (*else*)
  end; (*case*)
end; (*of procedure InitEnv*)

           (*************************************************)
           (********  Prints the environmental grid  ********)
           (*************************************************)
Overlay Procedure PrtEnv (var Grid : CellGrid; D1, D2 : Integer);
var
  i,j  .: Integer;
begin
writeln(lst,'');
writeln(lst,'');
writeln(lst,'              **********************************             ');
writeln(lst,'                 Description of Environment');
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
writeln(lst,'            **********************************             ');
writeln(lst,'');
writeln(lst,'Dimensions of environment are ',D1,' rows by ',D2,' columns.');
writeln(lst,'');
writeln(lst,'Distribution of cell types in the environment is:');
writeln(lst,'');
writeln(lst,'');
for i := 1 to D1 do                                   (***Print grid ***)
  begin
  write (lst,'       ');
  for j := 1 to D2 do
    write (lst,Grid[i,j]:2,' ');
  writeln (lst,'');
  end; (*for*)
end; (*of procedure PrtEnv*)

           (*****************************************************************)
           (********  Assign population parameters to each cell type ********)
           (*****************************************************************)
Overlay Procedure ReadCellParam (var Num : CellType; var P1, P1SD, P2, P2SD : CellPara
label
  label1,
  label2;
const
  Low     : CellType = 1;
var
  i, Dum  : CellType;
  answer  : Char;
  x       : Real;
  CellFile: Text;
  Name    : Word;
  TF      : Boolean;
begin
label2: clrscr;
writeln('Choose the method to enter the cell population parameters');
writeln('');
writeln('');
writeln('a.  Specified from disk file');
writeln('b.  Specified from keyboard');
writeln('');
writeln('q.  Quit');
writeln('');
label1: readln (answer);
case answer of
  'a': begin
       Name := '';
       GetFile (CellFile, Name);
       if Name = 'quit' then
         goto label2;
       reset(CellFile);
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
       for i := 1 to Num do
         begin
         readln (CellFile, Dum, P1[i], P1SD[i], P2[i], P2SD[i]);
         if Dum <> i then
           begin
           writeln('Cell type mismatch');
           writeln(i,' ',Dum,' ',P1[i],' ',P1SD[i],' ',P2[i],' ',P2SD[i]);
           write('Type any key to continue, or "q" to quit.  ');
           readln(answer);
           if answer = 'q' then
             goto label1;
           end; (*if*)
         end; (*for*)
       close(CellFile);
       end; (*'a'*)
  'b': begin
       clrscr;
       writeln('Population parameters for each cell are r, SD of r, K, SD of K');
       for i := 1 to Num do
         begin
         writeln('Enter the population parameters for cell type ',i);
         readln(P1[i], P1SD[i], P2[i], P2SD[i]);
         end; (*for*)
       write ('Any changes? (Y/N)   ');
       readln (Answer);
       while (Answer = 'y') or (Answer = 'Y') do
         begin
         repeat
           writeln ('Specify cell type number and parameter column number (row, column
           readln (i,Dum);
           CellRange (TF, i, Low, Num);
           case Dum of
             1: P1[i] := x;
             2: P1SD[i] := x;
             3: P2[i] := x;
             4: P2SD[i] := x;
             else begin
                  writeln ('No such parameter, try 1, 2, 3, or 4');
                  TF := True;
                  end; (*else*)
             end; (*case*)
         until TF = False;
         write ('Enter new value:   ');
         readln (x);
         write ('Any more changes? (Y/N)  ');
         readln (Answer);
         end; (*while*)
       end; (*'b'*)
  'q': begin
       writeln ('Quitting...');
       end; (*'q'*)
  else begin
       writeln ('Try again');
       goto label1;
       end; (*else*)
  end; (*case*)
end; (*of procedure SetCellParam*)


           (*****************************************)
           (********  Print cell parameters  ********)
           (*****************************************)
Overlay Procedure PrtCellParam (var Num : CellType; var P1, P1SD, P2, P2SD : CellParam
var
  i     : Integer;
begin
writeln(lst,'');
writeln(lst,'');
writeln(lst,'                  ***********************');
writeln(lst,'                      Cell Parameters');
writeln(lst,'                  ***********************');
writeln(lst,'');
writeln(lst,'Number of types of cells is ',Num);
writeln(lst,'');
writeln(lst,'Parameter values are:');
writeln(lst,'');
writeln(lst,'');
writeln(lst,'Cell Type    r     SD of r    K   SD of K');
writeln(lst,'');
for i := 1 to Num do
  writeln(lst,'    ',i,'     ',P1[i]:7:5,'  ',P1SD[i]:7:5,'  ',
              P2[i]:5:2,'  ',P2SD[i]:5:2);
end; (*of procedure PrtCellParam*)

           (*************************************************************)
           (********  Initializes and Prints Dispersal Parameters  ********)
           (*************************************************************)
Overlay Procedure InitDispParam (var MoveGrid,DistMat:RealGrid; var LR:Real:D1,D2:Inte
var
  LeaveRate     : Real;
  Diffusivity   : Real;
  Name          : Word;
           (********  Read Real Number    ********)
Procedure ReadReal (var Num : Real; Name : Word);
var
  TF    : Boolean;
begin
repeat
  write ('Enter the ',Name,': ');
  TF := False;
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
  {$I-}
  readln (Num);
  {$I+}
  if IOResult <> 0 then
    TF := True;
until TF = False;
end; (* of procedure ReadDisp *)
             (********  Calculates dispersal proportions  ********)
Procedure CalcMove (var Grid: RealGrid; D:Real;D1,D2:Integer);
const

  A1 = 0.278393;
  A2 = 0.230389;
  A3 = 0.000972;
  A4 = 0.078108;
var
  i,j     : Integer;
  G1      : Arr1;
  G2      : Arr2;
begin
D := 1/sqrt(2*D);
for i := 1 to D1 do
  begin
  G1[i] := (i-0.5)*D;
  if G1[i] < 20 then
    begin
    G1[i] := 1 + A1*G1[i] + A2*G1[i]*G1[i] + A3*G1[i]*G1[i]*G1[i] + A4*G1[i]*G1[i]*G1[
    G1[i] := 0.5/(G1[i]*G1[i]*G1[i]*G1[i]);
    end
  else G1[i] := 0.0;
  end;(*for*)
for i := 1 to D2 do
  begin
  G2[i] := (i-0.5)*D;
  if G2[i] < 20 then
    begin
    G2[i] := 1 + A1*G2[i] + A2*G2[i]*G2[i] + A3*G2[i]*G2[i]*G2[i] + A4*G2[i]*G2[i]*G2[
    G2[i] := 0.5/(G2[i]*G2[i]*G2[i]*G2[i]);
    end
  else G2[i] := 0.0;
  end;(*for*)
G1[0] := 2*(0.5 - G1[1]);
for i := 1 to D1-1 do
  G1[i] := G1[i] - G1[i+1];
G2[0] := 2*(0.5 - G2[1]);
for i := 1 to D2-1 do
  G2[i] := G2[i] - G2[i+1];
for i:= 1 to D1 do
  for j := 1 to D2 do
    Grid[i,j] := G1[i-1]*G2[j-1];
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
end;(* of procedure CalcMove *)
           (********  Calculates Distance Matrix  ********)
Procedure CalcDistMat (var Grid: RealGrid; D1, D2: Integer);
var
  ColCent, RowCent  : Integer;
  i, j              : Integer;
begin
ColCent :- (D1 div 2) + (D1 mod 2);
RowCent :- (D2 div 2) + (D2 mod 2);
for i :- 1 to D1 do
  for j :- 1 to D2 do
    Grid[i,j] :- sqrt(sqr(i-ColCent)+sqr(j-RowCent));

end;(*CalcDistMat*)
begin
Name :- 'leaving rate from site';                          (*Set leaving rate*)
ReadReal (LR, Name);
Name :- 'diffusivity (cell units)';                        (*Set diffusivity*)
ReadReal (Diffusivity, Name);
(*print leave rate and diffusivity*)
writeln (lst,'');
writeln (lst,'Leaving rate form each cell - ',LR:6:3);
writeln (lst,'Diffusivity (units of cell size) - ',Diffusivity:6:3);
CalcMove (MoveGrid, Diffusivity, D1, D2);                  (*Calculate movement prop
CalcDistMat (DistMat, D1, D2);
end;(*of procedure InitDispParam*)

           (************************************************)
           (********  Initializes the population grid  ********)
           (************************************************)
Overlay Procedure InitPop (var Grid : RealGrid; D1,D2:Integer; N:CellType; E:CellGrid;
label
  label1,
  label2;
var
  Dum, Dum1 : Real;
  i,j       : Integer;
  List,Name : Word;
  Num       : CellType;
  LFil,PFil : Text;
Procedure UniInit (var G : RealGrid; Val : Real; D1, D2 : Integer);
var
  i,j     : Integer;
begin
for i :- 1 to D1 do
  for j :- 1 to D2 do
    G[i,j] :- Val;
end; (* of procedure UniInit*)
begin
label2: clrscr;
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
writeln('Choose the way you want to initialize population density');
Writeln('by typing the appropriate letter');
writeln('');
writeln('a.  Uniform initial conditions (same starting density everywhere');
writeln('b.  Delta function at center of grid');
writeln('c.  Uniform on one cell type');
writeln('d.  Random uniformly on all cells');
writeln('e.  Random uniformly on one cell type');
writeln('f.  Specified for each cell from disk file');
writeln('g.  Specified for each cell from keyboard');
writeln('');
writeln('q.  Quit');
writeln('');
label1: readln (Answer);
clrscr;
case answer of
  'a': begin
       write('Enter the initial starting density:  ');
       readln(Dum);
       UniInit (Grid, Dum, D1, D2);
       end; (*'a'*)
  'b': begin
       Dum := 0.0;
       UniInit (Grid, Dum, D1, D2);
       write('Enter the initial starting density for center cell:  ');
       readln(Dum);
       i := (D1 div 2) + (D1 mod 2);
       j := (D2 div 2) + (D2 mod 2);
       Grid[i,j] := Dum;
       end; (*'b'*)
  'c': begin
       Dum := 0.0;
       UniInit (Grid, Dum, D1, D2);
       write('Enter the cell type:  ');
       readln(Num);
       Write('Enter starting density:  ');
       readln(Dum);
       List := 'List';
       Locate (Num, E, D1, D2, List, LFil);
       GetFile (LFil, List);
       reset (LFil);
       while not EOF(LFil) do
         begin
         readln (LFil, i, j);
         Grid[i,j] := Dum;
         end; (*while*)
       end; (*'c'*)
  'd': begin                          (***  rnadom assignment of cell types  ***)
       write('Enter range of starting densities (min, max):  ');
       readln(Dum,Dum1);
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
      for i := 1 to D1 do
        for j := 1 to D2 do
          Grid[i,j] := Dum + (Dum1 - Dum)*Random;
      end; (*'d'*)
  'e': begin                       (*** random assignment to one cell type ***)
      Dum := 0.0;
      UniInit (Grid, Dum, D1, D2);
      write('Enter cell type:  ');
      readln(Num);
      write('Enter range of starting densities (min, max):  ');
      readln(Dum, Dum1);
      List := 'List';
      Locate (Num, E, D1, D2, List, LFil);
      GetFile (LFil, List);
      reset (LFil);
      while not EOF(LFil) do
        begin
        readln (LFil, i, j);
        Grid[i,j] := Dum + (Dum1 - Dum)*Random;
        end; (*while*)
      end; (*'e'*)
  'f': begin                                 (***  from disk file  ***)
      Name := '';
      GetFile(Pfil, Name);
      if Name = 'quit' then
        goto label2;
      reset (PFil);
      for i := 1 to D1 do
        for j := 1 to D2 do
          read(PFil, Grid[i,j]);
      close (PFil);
      end; (*'f'*)
  'g': begin                                 (***  from keyboard  ***)
      clrscr;
      writeln ('Enter <cr> for each cell value');
      for i := 1 to D1 do
        begin
        writeln ('Enter the ',D2,' population densities for row ',i,':  ');
        for j := 1 to D2 do
          read (Grid[i,j]);
        writeln ('');
        end; (*for*)
      write ('Any mistakes? (Y/N)  ');
      readln (Answer);
      while (answer = 'Y') or (answer = 'y') do
        begin
        write ('Specify grid coordinates (row,collumn):  ');
        readln (i,j);
        write ('Enter new population density:  ');
        readln (Grid[i,j]);
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
         write ('Any more corrections? (Y/N)   ');
         readln (Answer);
         end; (*while*)
       end; (*'g'*)
  'q': begin
       writeln ('Quitting...');
       end; (*'q'*)
  else begin                                                   (***  mistake  ***)
       writeln ('Try again');
       goto label1;
       end; (*else*)
  end; (*case*)
end; (* of procedure InitPop *)

           (************************************************************)
           (********  Prints the initial population densities  ********)

           (************************************************************)
Overlay Procedure PrtIPop (var Grid : RealGrid; D1,D2:Integer; Ans:Char);
var
  i,j   : Integer;
begin
writeln(lst,'');
writeln(lst,'');
writeln(lst,'              **********************************             ');
writeln(lst,'                 Initial Population Density');
writeln(lst,'              **********************************             ');
writeln(lst,'');
case Ans of
  'a': writeln('Uniform initial conditions (same starting density everywhere');
  'b': writeln('Delta function at center of grid');
  'c': writeln('Uniform on one cell type');
  'd': writeln('Random uniformly on all cells');
  'e': writeln('Random uniformly on one cell type');
  'f': writeln('Specified for each cell from disk file');
  'g': writeln('Specified for each cell from keyboard');
  end; (*case*)
writeln(lst,'********** T = 0');
writeln(lst,'Initial population densities in each cell are:');
writeln(lst,'');
writeln(lst,'');
if (Ans = 'd') or (Ans = 'e') then
  begin
  for i := 1 to D1 do                                     (***Print grid ***)
    begin
    write (lst,'   ');
    for j := 1 to D2 do
      write (lst,Grid[i,j],' ');
    writeln (lst,'');
    end; (*for*)
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
  end (*if*)
else
  begin
  for i := 1 to D1 do                                   (***Print grid ***)
    begin
    write (lst,'   ');
    for j := 1 to D2 do
      write (lst,Grid[i,j]:6:3,' ');
    writeln (lst,'');
    end; (*for*)
  end; (*else*)
end; (*of procedure PrtIPop*)

            (*********************************************)
            (********  Initialize dispersal grid  ********)
            (*********************************************)
Overlay Procedure InitDisp (var Grid : RealGrid; D1, D2:Integer);
var

  i,j     : Integer;
begin
for i := 1 to D1 do
  for j := 1 to D2 do
    Grid[i,j] := 0.0;
end;

            (***********************************************************)
            (********  Calculate and print population statistics  ********)
            (***********************************************************)
Overlay Procedure PopStats (Grid1, Grid2, DistGrid: RealGrid; D1, D2, Time: Integer);
var
  ColCentroid, RowCentroid : Real;
  TotalPopSize             : Real;
  MSD                      : Real;
  MeanDist                 : Real;
  FarthestDist             : Real;
  i,j                      : Integer;
  Grid                     : RealGrid;
  RowSum                   : Arr1;
  ColSum                   : Arr2;
  Max                      : Real;
begin
TotalPopSize := 0;
MSD := 0;
MeanDist := 0;
FarthestDist := 0;
Max := 0;
for i := 1 to D1 do
  for j := 1 to D2 do
    begin
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
    Grid[i,j] := Grid1[i,j] + Grid2[i,j];
    TotalPopSize := TotalPopSize + Grid[i,j];
    MeanDist := MeanDist + DistGrid[i,j] * Grid[i,j];
    MSD := MSD + sqr(DistGrid[i,j]) * Grid[i,j];
    if Grid[i,j] > Max then
      Max := Grid[i,j];
    if Grid[i,j] > 0.001 then
      if DistGrid[i,j] > FarthestDist then
        FarthestDist := DistGrid[i,j];
    end;(*for*)
MeanDist := MeanDist / TotalPopSize;
MSD := MSD / TotalPopSize;
RowCentroid := 0;
for i := 1 to D1 do
  begin
  RowSum[i] := 0;
  for j := 1 to D2 do
    RowSum[i] := RowSum[i] + Grid[i,j];
  RowCentroid := RowCentroid + i * RowSum[i];
  end;(*for*)
RowCentroid := RowCentroid / TotalPopSize;
ColCentroid := 0;
for j:= 1 to D2 do
  begin
  ColSum[j] := 0;
  for i := 1 to D1 do
    ColSum[j] := ColSum[j] + Grid[i,j];
  ColCentroid := ColCentroid + j * ColSum[j];
  end;(*for*)
ColCentroid := ColCentroid / TotalPopSize;
writeln (lst,'');
writeln (lst,'');
writeln (lst,'');
writeln (lst,'********** T = ',Time);
writeln (lst,'Total Population Size =  ',TotalPopSize:8:3);
writeln (lst,'Farthest Distance Moved (0.001 density threshold) = ',FarthestDist:8:3);
writeln (lst,'Mean Distance Moved = ',MeanDist:6:3,'   MSD = ',MSD:8:3);
writeln (lst,'Center of population is at ',RowCentroid:5:2,ColCentroid:5:2);
writeln (lst,'');
write(lst,' . is empty (0.001 threshold), () is up to 0.0001X, ++ is up to 0.001X,');
write(lst,' YY is up to 0.01X, SS is up to 0.1X, and ## is up to Maximum Density = ',M
writeln(lst,'');
for i := 1 to D1 do
  begin
  write (lst,'   ');
  for j := 1 to D2 do
    begin
    if Grid[i,j] < 0.001 then
      write(lst,' .')
    else
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
      if Grid[i,j] < 0.0001*Max then
        write(lst,'()')
      else
        if Grid[i,j] < 0.001*Max then
          write(lst,'++')
        else
          if Grid[i,j] < 0.01*Max then
            write(lst,'YY')
          else
            if Grid[i,j] < 0.1*Max then
              write(lst,'SS')
            else
              write(lst,'##');
    end;(*for*)
  writeln (lst,'');
  end; (*for*)
end; (*of procedure PopStats*)

            (********************************************************)
            (******** Increase populations within each cell  ********)
            (********************************************************)

Procedure Increase (var PopnGrid, DispGrid: RealGrid; r,rSD,K,KSD:CellParam;LR:Real;D1
label
  skip1;
var
  i,j   : Integer;
  Increase, MaxPop, Vac, New, Pop : Real;
begin
for i := 1 to D1 do
  for j := 1 to D2 do
    begin
    if (PopnGrid[i,j] > 0) or (DispGrid[i,j] > 0) then
      begin
      Increase := Normal(r[EnvironmentGrid[i,j]], rSD[EnvironmentGrid[i,j]]);
      MaxPop := Exp(Normal(K[EnvironmentGrid[i,j]], KSD[EnvironmentGrid[i,j]]));
      PopnGrid[i,j] := PopnGrid[i,j] + DispGrid[i,j];
      Vac := Limit(MaxPop, MaxPop - PopnGrid[i,j]);
      New := Limit(Vac, Increase*PopnGrid[i,j]*Vac/MaxPop);
      goto skip1;
      DispGrid[i,j] := LR * New;
      PopnGrid[i,j] := PopnGrid[i,j] + New - DispGrid[i,j];
      skip1: Pop := PopnGrid[i,j] + New;
      DispGrid[i,j] := Pop * LR;
      PopnGrid[i,j] := Pop - DispGrid[i,j];
      end; (*if*)
    end; (*for*)
end;(* of procedure Increase *)

            (***********************************************)
```

Appendix 6: Simple Spread Program with Modifications (continued).

```
            (********  Moves dispersing individuals  ********)
            (**********************************************)
Procedure Move (var DispGrid: RealGrid; MoveGrid:RealGrid; D1, D2: Integer);
var
  i,j,k,l  : Integer;
  DumGrid  : RealGrid;
begin
for i := 1 to D1 do
  for j := 1 to D2 do
    DumGrid[i,j] := 0.0;
for i := 1 to D1 do
  for j := 1 to D2 do
    begin
    for k := i to D1 do
      for l := j to D2 do
        DumGrid[k,l] := DumGrid[k,l] + DispGrid[i,j] * MoveGrid[k-i+1,l-j+1];
    for k := i to D1 do
      for l := j-1 downto 1 do
        DumGrid[k,l] := DumGrid[k,l] + DispGrid[i,j] * MoveGrid[k-i+1,j-l+1];
    for k := i-1 downto 1 do
      for l := j to D2 do
        DumGrid[k,l] := DumGrid[k,l] + DispGrid[i,j] * MoveGrid[i-k+1,l-j+1];
    for k:= i-1 downto 1 do

      for l := j-1 downto 1 do
        DumGrid[k,l] := DumGrid[k,l] + DispGrid[i,j] * MoveGrid[i-k+1,j-l+1];
    end;
for i := 1 to D1 do
  for j := 1 to D2 do
    DispGrid[i,j] := DumGrid[i,j];
end;(* of procedure Move*)


(*******************************************************************)
(*********************     Start Spread    ************************)
(*******************************************************************)
begin

(***  Initialization of Environment   ***)
ReadNoCellType (NoCellType);                           (*Read Number Cell Types*
InitEnv (NoCellType, EnvironmentGrid, D1, D2);         (*Initialize Environment*
goto skip1;
PrtEnv (EnvironmentGrid, D1, D2);                      (*Print Environment*)
skip1:;

(***  Initialization of Parameters  ***)
ReadCellParam (NoCellType, r, rSD, K, KSD);            (*Read Cell Parameters*)
PrtCellParam (NoCellType, r, rSD, K, KSD);             (*Print Cell Parameters*)
InitDispParam (MoveGrid, DistMat, LeaveRate, D1, D2);  (*Init Dispersal Paramete
```

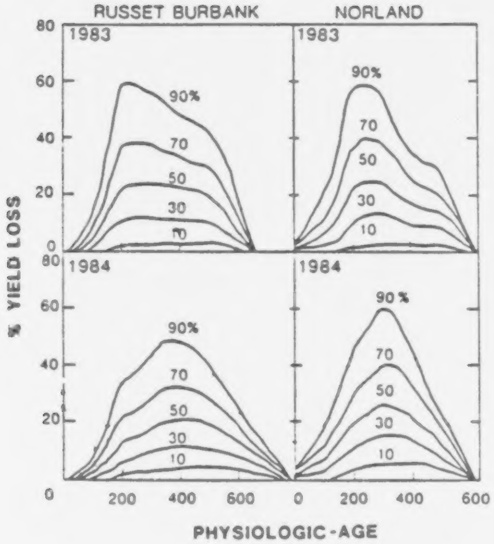
Appendix 6: Simple Spread Program with Modifications (continued).

```
(*** Initial Conditions for Population ***)
InitPop (PopnGrid, D1, D2, NoCellType, EnvironmentGrid, A1); (*Initialize Popn Density
if A1 = 'q' then                                         (*Skip Dynamic Section*)
  goto bottom;
goto skip2;
PrtIPop (PopnGrid, D1, D2, A1);                          (*Print Initial Pop Den*)
skip2:;
InitDisp (DispGrid, D1, D2);                             (*Initialize Dispersal*)

(*** Dynamic Section ***)
for Time := 1 to 10 do
  begin
  Increase (PopnGrid, DispGrid, r, rSD, K, KSD, LeaveRate, D1, D2);
  Move (DispGrid, MoveGrid, D1, D2);
  if (Time mod 5) = 0 then
    PopStats (PopnGrid, DispGrid, DistMat, D1, D2, Time);
  end;(*for*)

bottom: writeln('done');
end. (*of Spread*)
```

Appendix 7. Potato Yield Loss by Frost

Figure A7-1. Simulated yield loss of potato cultivar Russet Burbank when defoliated with varying intensity at different growth stages (from Johnson et al., 1985). Effects of defoliation are probably overestimated by model. Average yield of Russet Burbank is about 225 cwt/acre (Johnson et al., 1985), and farm value is between $4.00 and $5.00/cwt. Therefore a 10 percent yield loss is about 22.5 cwt/acre or $90.00 to $124.00/acre.

Fig A7-1



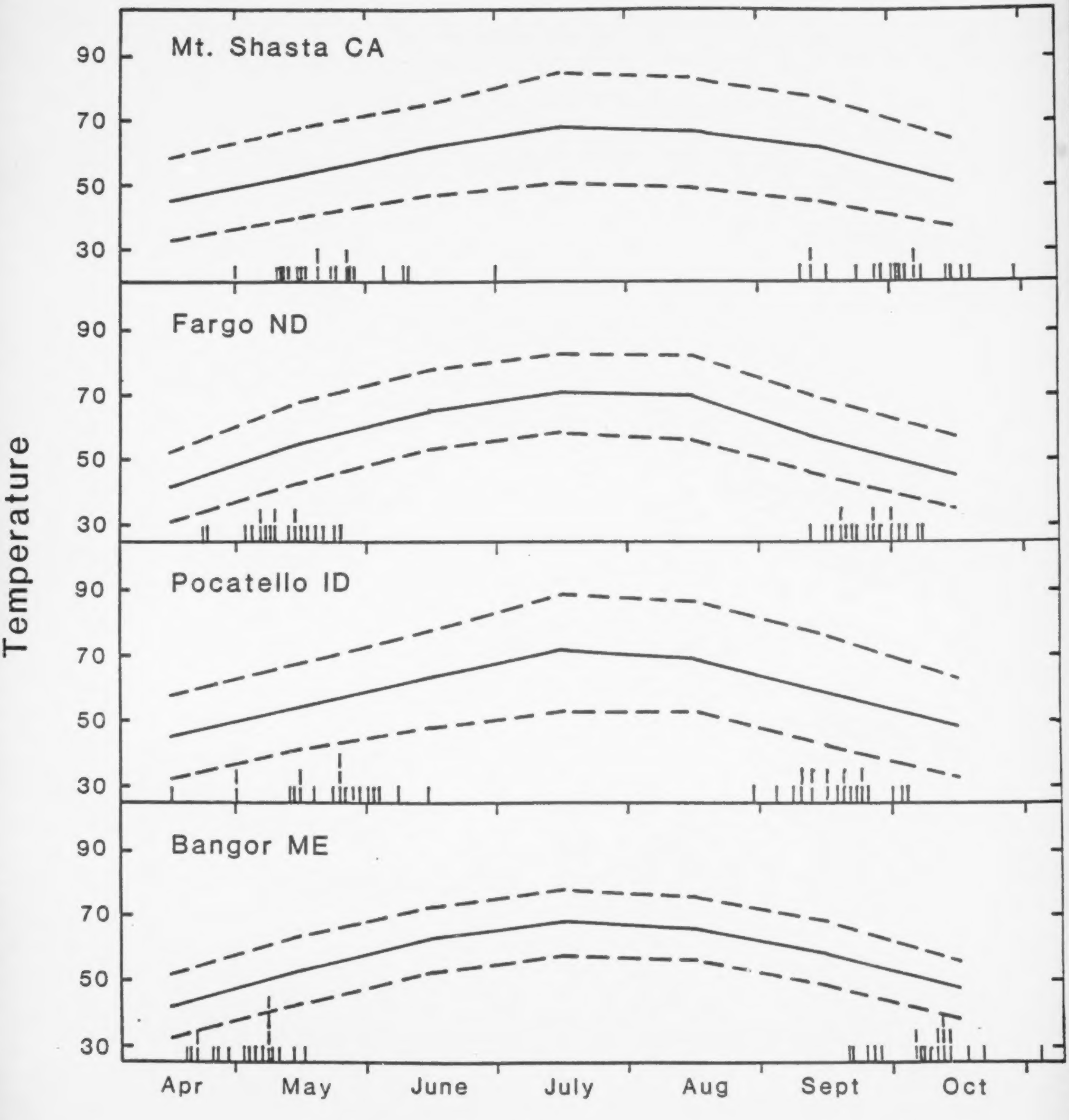
RUSSET BURBANK
NORLAND
1983
1983
1984
1984
90%
70
50
30
10
90%
70
50
30
10
90%
70
50
30
10
90 %
70
50
30
10
80
60
40
20
0
80
60
40
20
0
% YIELD LOSS
200
400
600
0
200
400
600
PHYSIOLOGIC-AGE

Table A7-1. Simulated yield loss, probability such as yield loss or greate will occur, and the time of the limiting frost (s:spring; f:fall) at Pocatello, ID and Mt. Shasta, CA. Yield loss was estimated with the 90% defoliation curve in Figure A7-1.

	Yield loss (percent)	Probability of Yield Loss or Greater	Expected Yield Loss (percent)	Time of Yield-Limiting Frost
Pocatello, ID				
June 15 - 50% emergence	29	0.05	1.45	F
	18	0.25	4.50	F
	12	0.50	6.00	F
	8	0.75	6.00	F
	2	1.00	2.00	F
June 1 - 50% emergence	18	0.005	0.09	S/F
	15.5	0.05	0.78	F
	6.5	0.25	1.63	F
	0	1.00	0.00	-
May 20 - 50% emergence	25.5	0.0025	0.06	S/F
	20	0.05	1.00	S
	10	0.1	1.00	S
	3	0.3	0.90	S
	0	1.0	0.00	-
Mt. Shasta, CA				
June 15 - 50% emergence	29.5	0.0025	0.07	S/F
	21.5	0.05	1.08	F
	12.5	0.25	3.13	F
	6.5	0.5	3.25	F
	3	0.75	2.25	F
	0	1.0	0.00	-
June 1 - 50% emergence	41	0.025	1.03	S/F
	33	0.05	1.65	S
	3	0.15	0.45	S
	2	0.25	0.50	F
	0	1.0	----	-
May 20 - 50% emergence	40	0.05	2.00	S
	15	0.1	1.50	S
	10	0.15	1.50	S
	5	0.2	1.00	S
	3	0.3	0.90	

Figure A7-2. Thirty year average maximum and minimum temperatures at 4 locations. Average of maximum and minimum temperatures also shown. Distribution of spring and fall frosts are indicated along the abscissa.

Fig A4-6


Mt. Shasta CA
Fargo ND
Pocatello ID
Bangor ME
Temperature
90
70
50
30
Apr
May
June
July
Aug
Sept
Oct

Limitations in Analysis

1. Analysis is restricted to one cultivar, Russet Burback. This is not as restrictive as it might seem. Russet Burbank is the potato of choice in Idaho and yields well. Growers could tolerate a 22 percent yield loss to Russet Burbank before switching to the more rapidly maturing Norland (600 physiologic units to maturity).

2. Extrapolation of model. The model was constructed for use in Minnesota to model yield loss from pathogen or insect induced defoliation. There may be idiosyncracies in this formulation which restrict generalizations to other geographic conditions and for frost induced defoliation. The model is still being developed and improved to model Minnesota conditions more accurately.

3. Any frost completely destroys haulm. Light frosts might not kill plants, more severe frosts might only kill canopy leaves, and shoots might resprout after a severe frost.

4. Weather data representative. Pocatello, ID is less than 10 km away from important experimental test plots of potatoes. Mt. Shasta may not be representative of its region. Potatoes grown in valleys may be more vulnerable to frosts.

5. Independence of frosts and temperatures. Potato growth is modeled as if average temperatures always prevailed, and growing temperature is independent of frost date. In addition, the date of the last spring frost is assumed to be independent of the date of the first fall frost. It would be more realistic to simulate each year seperately, since a cold year might have a late spring frost and an early fall frost.

6. Temperature and frost distributions are representative. This assumes that the past 30 years are representative of the next 30 years. Since there are climatic cycles on this time scale, local climate may vary. Currently, northern United States is becoming cooler.

7. Economic feasibility is determined by expected benefits. Growers may not be able to average benefits over long periods of time, or may discount future benefits. In addition, a farm budget analysis or a regional competition model would be a more appropriate analysis.

An Assessment of the Impact of Large-Scale Applications of Ice-minus Bacteria and other Procedures Designed to Decrease Population Sizes of Ice-Nucleation-Active Bacteria on Crops.

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CHAPTER I. EXECUTIVE SUMMARY.

A. Overall conclusions.

There is a distinct probability that removal of INA bacteria from large areas of cropland will affect precipitation formation.

Evidence currently available is insufficient to allow us to determine exactly how large the effect will be.

Three of the four possible technologies that might be used to prevent frost injury to crops were indistinguishable with regard to which might cause the largest effect on bacterial ice nuclei.

Thus, the major issue ought to be not whether or not GEMs should be used to protect crops from frost, but whether or not technologies that decrease numbers of bacterial ice nuclei ought to be used to protect plants from frost injury.

Current information is insufficient to allow a definitive answer to even this question.

Most of the uncertainty associated with the relationship between INA bacteria and atmospheric processes, and with the extent to which any given treatment is likely to affect INA bacterial populations beyond the treated area can be resolved by a modest amount of research. This research should be performed before a final decision is made as to whether any of these technologies should be implemented on a large scale.

B. Summary.

Large economic losses to agricultural crops due to frost injury occur frequently in the U. S. These provide substantial economic incentive for development of technologies that may decrease the frost hazard. Frost injury requires formation of ice before plant tissues are affected. Ice does not form at the temperatures at which frost injury occurs in the absence of ice nuclei. The most efficient, most abundant ice nuclei present on most plants are ice nucleation active (INA) bacteria. Other ice nuclei are present, but in most cases, they are active at temperatures that may be as much as 3 C lower than are bacterial ice nuclei.

Five species of bacteria are known to be active as ice nuclei (Pseudomonas syringae, P. viridiflava, P. fluorescens, Erwinia herbicola, Xanthomonas campestris pathovar translucens). All are frequent residents on leaves of plants and, indeed, only the conifers as a group have been found not to carry measurable populations of INA bacteria. Among the five species, P. syringae appears to be the most efficient ice nucleus and probably the most important with regard to frost injury. The current (and rather limited) understanding of the population biology of INA bacteria is based primarily on studies on P. syringae. The population densities of P. syringae and the proportion of total epiphytic bacterial populations that may be composed of the bacterium are dependent on a number of interacting factors including host genotype, geographic location, and weather conditions. However, no intensive and extensive studies have been conducted to assess the quantitative influence of these various factors on populations of INA bacteria. Population densities of P. syringae can change dramatically and rapidly (over 1000 fold in 24 hours) with time. Large population increases have usually been preceded by rain. Epiphytic bacteria are dispersed into the air as aerosols. INA bacteria are likely to be carried aloft during dry sunny days. They may be redeposited on plants in low numbers from aerosols as far as 60 km downwind from the source, or by rain.

Plants that are likely to support growth of INA bacteria may occupy as much as 1,855 million acres, or about 97.5% of the area of the lower 48 states of the U. S. Cropland is about 474 million acres, or 24.9% of the area of the same states. Frost sensitive crops are grown on over 206 million acres, or 10.8% of the same area. Regional differences in acreage planted to frost sensitive crops are very large, ranging from about 0.04% of Nevada to 61% of Iowa. Although it is not likely that all of the land treated to frost sensitive crops will be treated to remove bacterial ice nuclei nationwide, on a regional or local basis, treatment of all or most of the land devoted to production of these crops seems more likely. It is conceivable that, if appropriate technologies become available, areas the size of entire counties, perhaps entire states, may become denuded of INA bacteria. The impact of reduction of the temperature at which crops are damaged by frost from -2.2 C to -4.4 C can be illustrated by comparing the probabilities of frost events at these two temperatures. If we take the day on which there is a 50% probability of frost at -2.2 C (both spring and fall) for our basis of comparison, the average probability of a -4.4 C frost is only 16% for 180 stations nationwide. Thus, most of the nation's producers of frost sensitive crops will find such technologies tempting to consider.

Since dispersal of epiphytic bacteria is expected, some degree of establishment and growth of these bacteria on the leaves or plants to which they spread is expected. Thus, the area on which population sizes of INA bacteria are affected may be somewhere between the actual area treated for frost protection and some larger area that might be viewed as the limit to which epiphytic bacterial populations on other plants are affected by spread of bacteria from the treated area. Two scenarios were proposed, the "direct" scenario, in which the effect of a given treatment is limited to the treated area, and the "dispersal" scenario, in which an area about twice the size of the treated area is affected due to bacterial spread. The choice of double the apparent area is arbitrary, and probably much larger than likely, due to selection of each plant species for the bacteria that grow upon it.

There are a number of ways that have been demonstrated as having potential for protecting crops against frost injury by inactivating or eliminating bacterial ice nuclei.

Ice nucleation inhibitors
Bactericides
Antagonistic bacteria
Plant genetics.

Each of the four potential technologies was evaluated with respect to the likelihood that it will be implementable, and the duration and area of its effect. Ice nucleation inhibitors are likely to affect only the area to which they are applied, and only at times when frost is imminent. Thus, they are likely to affect the smallest number of bacterial ice nuclei. They are also the least likely to be developed for commercial use, largely for technical reasons. Bactericides, particularly those with a relatively narrow spectrum, should they be developed, were found to have an effect for a longer period, and probably to affect more than just the treated area. Effective bactericides will probably be utilizable on many, or most crops, and thus may be applied to a relatively large area, if they become available. Although one material is currently registered for use for frost protection, we do not expect to see any effective bactericides on the market in the near future. There currently is commercial activity directed toward development of genetically engineered microorganisms (GEMs) for frost protection by competitive exclusion of INA bacteria. GEMs will probably spread beyond the treated area to some degree, and may provide adequate frost protection on a number of crops. They will probably have to be applied long before frost, several months in some cases. The area to which GEMs are applied may be limited by plant - bacterial specificity. Plant genetics can be used to develop cultivars of crops that select against INA bacteria. Cultivars of this type may provide the most reliable and least expensive protection against frost injury of all of the technologies. The area planted to resistant cultivars will be affected all season long, and substantially more than this planted area may be affected. Implementation of this technology will be slow, and limited with respect to crop.

With current data, we are not able to determine which of the possible technologies is likely to have the greatest impact on total numbers of bacterial ice nuclei, although ice nucleation inhibitors will probably have the least effect.

Ice nuclei constitute one of several factors determining the efficiency of clouds in producing precipitation. The weight of their importance varies from none to dominant, depending on cloud type and on other meteorological conditions.

The origins of atmospheric ice nuclei must be considered to be mostly unknown. However, in areas extensively covered by vegetation, it is possible that INA bacteria may be an important fraction of the total ice nucleus population at small supercoolings (i.e., near 0 C). If organic ice nuclei in soils are shown to be derivatives of the INA bacteria on plants, as some evidence now suggests, then the importance of INA bacteria as sources of atmospheric ice nuclei is strongly amplified. These suppositions are subject to experimental verification, but the appropriate measurements have not yet been made.

Bacterial ice nuclei, and their derivatives residing in the soil, are most likely to have an impact on the development of precipitation in convective clouds. The entry of the bacterial or soil nuclei into these clouds is effectively ensured by the updrafts producing the cloud, and the concentrations of these nuclei can be high enough to exert a dominating influence on the total ice nucleus concentrations in the updrafts. These expectations are also subject to direct experimental verification.

If bacterial ice nuclei constitute the majority of the ice nuclei present in certain storm systems, reduction of these nuclei to the extent they must be decreased to avoid frost injury would essentially eliminate any possible role the ice nuclei could play in the clouds.

The direct or indirect removal of INA bacteria from contiguous areas of a few hundred acres appears to constitute the threshold beyond which the development of precipitation might become affected. Beyond this threshold, the anticipated effect on convective rainfall would be roughly proportional to the fraction of land area over which nucleus concentrations are reduced. For the regions with large fractions of treated areas, a further effect, displaced a hundred or more miles downwind, is likely to occur.

The most frequent situation, in which crops occupy less than 1/2 of the total land area, where the patches of crops are no greater than a few kilometers in size, and where the non-crop land carries populations of INA bacteria comparable to the crops, treatments to remove or reduce the activity of the INA bacteria on the crops will impact the flux of ice nuclei available to clouds to a small enough extent that the normal precipitation amount of the region is not likely to be influenced to a noticeable degree. There may be reductions in the frequency of light to moderate showers. There may also be changes, either increases or reductions, in the frequency or sizes of hail.

The meteorological impacts of crop treatments become more serious if the INA bacterial populations of non-crop areas also become reduced. The origin of the organic ice nuclei in soils is not known. Even greater meteorological impacts are expected if the organic ice nuclei in soils become reduced as a result of treatments over several successive years.

Consideration of the likely effects on precipitation of frost prevention technologies in two very different climates have lead us to the following conclusions. First, although there may be an effect on the average amount of precipitation, the amount of this reduction will be so small relative to the normal variability in precipitation that it will be difficult to measure. Second, the effect on the frequency of precipitation during dry periods particularly, from small systems, may be larger than the effect on average precipitation. Third, changes in the proportion of precipitation associated with heavy showers or light showers, and of hail are likely to occur. Finally, although the largest sources of uncertainty in our analysis reflect large gaps in what is know about this important area, a more thorough analysis of the effect of decreasing numbers of ice nuclei on precipitation production in both areas is possible using current data.

CHAPTER II. BACKGROUND.

This chapter provides a brief summary of the relationship between bacterial ice nucleation and frost injury, and the various technologies that have been proposed to decrease temperatures at which crops are at risk to frost injury by decreasing numbers of bacterial ice nuclei present on the plants.

For purposes of this report, we will use frost injury to refer to injury to plants that are not able to tolerate extracellular ice within their tissues. This is a rather narrow definition. However, it is precisely these plants to which bacterial ice nucleation is likely to be important for causing frost injury. Literature definitions of frost injury may or may not be more inclusive.

A. Ice nucleation and frost injury to crop plants.

Ice nucleation is necessary for frost injury. Without ice there is no injury. Without heterogeneous ice nuclei water will supercool; that is, it will remain in the fluid state to temperatures approaching -40 C. It is the first ice nucleation event associated with each plant part that is critical for frost injury. Once ice is present on a leaf or flower, crystallization of supercooled water will continue as long as heat is removed and the temperature stays at or below 0 C. Additional ice nuclei have no effect. Thus, it is always the most efficient ice nucleus, that is, the one that is active at the warmest temperature that actually initiates the damage process.

Our understanding of the role of ice nucleation active (INA) bacteria in frost injury to plants has grown from the original observation that INA bacteria increased frost injury to growth-chamber grown corn seedlings at -4 to -5 C (Arny et al., 1976). Since that time, bacterial ice nuclei have been shown to be the major source of ice nuclei active at temperatures above -5 C associated with many crop plants (Lindow et al., 1978a). For these plants, the amount of injury, in both field and growth chamber, has been positively correlated with the population sizes of INA bacteria present on the plants (Lindow et al., 1978b,c, 1982b, 1983a). Since not every INA bacterial cell is active as an ice nucleus at any given time and temperature (discussed in detail in chapter III), Lindow (1982c) has demonstrated that frost injury is also positively related to numbers of bacterial ice nuclei on plant parts. The relationship between INA bacteria and frost injury has been reviewed by Lindow (1982a, 1983).

Ice nuclei are intrinsic to plants themselves. A search for abundant natural sources of ice nuclei led to the observation that decaying leaves were excellent sources of highly efficient ice nuclei (Schnell and Vali, 1972, 1973, 1976; Vali et al., 1976). An INA _P_. _syringae_ was isolated from leaf litter (Maki et al., 1974). Thus, some of the leaf-derived nuclei may actually be of bacterial origin. However, ice nucleation occurs at about -5 or -6 C in seedling corn or oat plants in the absence of INA bacteria (Hirano et al., 1985). Thus, ice nucleation attributable to these plants appears to begin to occur at temperatures lower than those observed for bacterial ice nuclei. Recent reports by Ashworth et al., (1985a,b) and Gross et al., (1984; Proebsting et al., 1982; Andrews et al., 1986) document the occurrence of ice nucleation associated with certain woody plants at temperatures as warm as -2 C, the range in which bacterial ice nuclei are thought to be important. Neither *P. syringae*, nor any other known INA bacterium could be isolated from these plants. The material was not axenic, however. Thus, the extent to which the most active ice nucleus associated with any given plant or plant part may be bacterial is in doubt for some woody plants. Anderson and Ashworth (1985) have shown low frequencies of similar warm temperature ice nuclei in tomatoes. However, natural frost injury to tomatoes was attenuated by procedures that decreased either numbers or activities of bacterial ice nuclei (Hirano et al., 1985; Lindow et al., unpublished). The bacteria responsible for ice nucleation, and their association with plants is discussed in some detail in chapter III.

B. The potential for protection against frost injury that may be achieved by altering population sizes of ice nucleation active bacteria.

Inasmuch as ice nucleation does not occur until approximately -5 C or below in many, perhaps most plants, in the absence of INA bacteria, and does occur at -2 C or above in the presence of INA bacteria, the potential exists to lower the temperature at which ice will form and hence frost injury occur by as much as 3 C by excluding or eliminating INA bacteria from plants.

C. Potential means by which INA bacteria or bacterial ice nucleation may be altered.

Four different approaches have been used to attempt to decrease numbers of bacterial ice nuclei associated with crops (Three of which have been reviewed by Lindow, 1982b). First, bactericides have been applied to kill bacteria, or prevent their growth. Another chemical means of protection of plants against frost has been to spray them with materials that inactivate bacterial ice nuclei without necessarily killing the bacterial cells. Materials that act in this way have been termed ice nucleation inhibitors. Biological control of frost injury has been achieved by addition of non-ice nucleating bacteria that compete with wild type ice nucleating bacteria on leaves and flowers. Host plant genetics can be used to alter population sizes of ice nucleating bacteria. The degree of frost protection afforded by each of these alternatives has been limited in the field to date. The likelihood and impact of their future success, and reasons for some of their limitations are discussed in chapter V.

D. Current (proposed) experiments to use genetically engineered microorganisms (GEMs) that lack the gene for ice nucleation to protect crops against frost injury by competition with wild type INA bacteria.

Recombinant DNA technology has been used to construct mutants of Pseudomonas syringae and Pseudomonas fluorescens that lack the ability to nucleate supercooled water at temperatures where bacterial ice nucleation is likely to be important in inciting frost injury (Orser et al., 1985). Drs. Steven E. Lindow and Nicholas J.Panopoulos, Department of Plant Pathology, University of California, Berkeley, and scientists at the Oakland, California-based company, Advanced Genetic Sciences have demonstrated the potential application of these ice-minus mutants as agents for biological protection of certain plant species against frost injury. Because the ice-minus mutants were derived from wild type ice-plus strains by genomic alteration using recombinant DNA procedures (i.e., site-directed deletion mutagenesis), testing of their efficacy as biological control agents has been restricted to 'closed' environments provided by green houses and growth chambers. However, research has progressed to the point where field-testing must be conducted to determine whether, indeed, these genetically-engineered ice-minus strains of *P. syringae* and *P. fluorescens* can be successfully employed to protect sensitive plants against frost injury.

The field trial proposed by Drs. Lindow and Panopoulos involves the application of each of two ice-minus strains of *P. syringae* to potato plants (Lindow, 1985; Federal Register 48:24548-24553, 1 June 1983). One of the parental strains was isolated from healthy potato leaves (Tulelake, CA), the other from healthy citrus leaves (near Exeter, CA). The proposed experimental plot area would encompass approximately 0.16 ha of potato plants at the University of California Agricultural Experiment Station, Tulelake, California.

The field test proposed by scientists at Advanced Genetic Sciences also involves each of two ice-minus deletion mutant strains (Federal Register 50:33841 21 Aug 1985). The parental strains are ice-plus *P. syringae* and *P. fluorescens* originally isolated from healthy strawberry plants. The target crop for these experiments is strawberry plants.

CHAPTER III. CURRENT STATUS OF OUR UNDERSTANDING OF THE POPULATION BIOLOGY OF INA BACTERIA.

A description of the taxonomy and habitat distribution of the five identified ice nucleation active bacterial species and the frequency with which the INA phenotype is expressed among strains of known INA bacterial species and among epiphytic bacteria in general are presented in this chapter. In addition, the distribution of population densities of INA bacteria on leaf surfaces and what is known abut the magnitude and manner in which these densities are likely to change in response to the physical (i.e., weather) and biological (i.e., host plant, introduced bacteria) environments are also discussed. Finally, what is known about the aerobiology (dispersal) of INA bacteria from plant surfaces is discussed.

A. The bacterial species that are known to be ice nucleation active.

At the present time, five species of bacteria belonging to three genera (*Pseudomonas*, *Erwinia*, and *Xanthomonas*) are known to be ice nucleation active. They are: *Pseudomonas syringae* van Hall, *Pseudomonas fluorescens* Migula, *Pseudomonas viridiflava* Dowson, *Erwinia herbicola* Dye, and *Xanthomonas campestris* (Dowson) pathovar *translucens*. These bacteria are gram-negative, flagellated rods. The Pseudomonads and Xanthomonads are strict aerobes; whereas, the Erwiniae are facultative anaerobes.

The species *P. syringae* has been subdivided by Plant Pathologists into 41 different subgroups, termed pathovars (pv.), primarily on the basis of the plants on which they cause disease (Dye et al., 1980; Palleroni, 1984). Thus, the host range differs among the pathovars. For example, strains of *P. syringae* pv. *syringae* are pathogenic to lilac, bean, wheat, and stone fruit trees including plum and cherry; whereas *P. syringae* pv. *glycinea* appears to be restricted as a pathogen to soybean. No susceptible host plant has been identified for some *P. syringae* strains. Such putative non-pathogenic strains were used as the parental INA strains isolated from potato and citrus that were used to construct the ice-minus deletion mutants (Lindow, 1985). The proportion of *P. syringae* strains that is ice nucleation active is discussed in part B of this chapter.

P. viridiflava is closely related to *P. syringae* on the basis of similar physiological and nutritional characteristics (Billing, 1970; Palleroni, 1984). *P. viridiflava* can be distinguished from *P. syringae* by its pectolytic ability and its inability to utilize sucrose. Although *P. viridiflava* has been isolated from diseased tissue of a range of plant species, it is generally viewed as a weak, opportunistic phytopathogen (cf. Jones et al., 1984).

Subdivisions (called biovars) also exist within the *P. fluorescens* group. Five biovars are currently recognized (Palleroni, 1984). The characterized ice nucleation active *P. fluorescens* strains belong to either Biovar I (strawberry isolate of Lindemann, AGS) or Biovar V (Maki and Willoughby, 1978; Fall and Schnell, 1985). Biovar I is considered to be representative of the species; whereas Biovar V is regarded as a heterogeneous group of miscellaneous strains that do not fit into the other biovars.

The taxonomy of the INA members of the Enterobacteriaceae is not entirely clear (Starr et al., 1981; Brenner, 1984; Lelliott and Dickey; 1984). One strain of E. herbicola has been adequately identified (Lindow et al., 1978b). Other strains that appear to be quite similar to this one are frequently found on leaves and other aerial plant parts. At least one INA bacterium in this group has been reported that apparently is not an E. herbicola, however (Levin et al., 1980; Yankofsky et al., 1981). Indeed, the genera Enterobacter and Erwinia are rather variable, and not well characterized. At any rate, ice nucleation is a moderately infrequent property of the strains that are probably E. herbicola. There is one report that E. stewartii increases the frost sensitivity of corn in a way that would imply that it, too is INA (Wallin et al., 1979). Direct tests of ice nucleation activity have not been reported for this strain.

The genus Xanthomonas includes bacteria that are pathogenic to a wide range of plant species (Dye et al., 1980; Bradbury, 1984). Similar to the pathovar designation within the P. syringae group, the species X. campestris has been subdivided into 125 recognized pathovars. The known ice nucleation active Xanthomonas strains have been identified as X. campestris pv. translucens. The pathogenic host range of this pathovar appears to be restricted to members of the Gramineae, including barley and wheat.

Although these are the only bacteria known (to us) to be ice nucleating at temperatures warm enough to pose a frost hazard to plants, the numbers of bacterial strains that have been tested for this property are very small. Attempts to identify all of the INA bacteria present on a given crop in several locations have not resulted in more species of INA bacteria. However, it is quite possible that the methods used are only suitable for detecting the species that have been found. In the case of only a few crops, and to our knowledge, in only two areas (southern Wisconsin and California), has a rigorous attempt succeeded to show that the identifiable INA bacteria were quantitatively responsible for all of the ice nucleation that could not be attributable to the plants themselves (Hirano et al., 1985; Lindow, 1982c; Lindow et al.,1978a). In other areas, notably California, it has been clearly shown that the numbers of identifiable INA bacteria were significantly correlated to the extent of frost injury at a particular temperature on several crops. The presence of ice nuclei active at -2 C in woody plants that do not contain known INA bacteria (Ashworth, et al., 1985a,b; Gross et al., 1984; Proebsting et al., 1982; Andrews et al., 1986), illustrates the fact that we do not know the sources of all of the efficient ice nuclei associated with plants in nature.

B. The relative proportions and distributions of INA and non-INA phenotypes.

1. Among species known to have some INA strains

Pseudomonas syringae. The ice nucleation activity of 348 strains of _P. syringae_, obtained from the National Collection of Plant

Pathogenic Bacteria (NCPPB), Harpenden, England was determined at -5.5 C and -10 C by Hirano et al. (1978). The strains were originally isolated from diseased tissue of a diverse range of plant species from different geographic regions in the world. Approximately 45% of the strains were active as ice nuclei at -5 C, 5% were not active at -5 C but were active at -10 C, and 51% were inactive at both temperatures. All or nearly all of the strains designated as _P_. _syringae_ pv. _syringae_ isolated from bean, Citrus spp., cucurbits, lilac, pea, pear, and poplar were active at -5 C; whereas, none of the strains designated as _P_. _syringae_ pv. _savastanoi_ from olive, _P_. _s_. pv. _tabaci_ from tobacco, _P_. _s._ pv. _tomato_ from tomato or _P_. _s_. pv. _morsprunorum_ from _Prunus_ species were active as ice nuclei even at temperatures as low as -10 C. A number of pathovars, from a range of plants were intermediate in the frequency with which ice nuclei were produced. Hence, the proportion of _P_. _syringae_ strains that are active as ice nuclei appears to depend, to a substantial extent, on the plant species from which they are isolated. This is consistent with the frequent observation that the _P_. _syringae_ associated with a particular plant belong to the pathovar that causes disease on that plant. Thus, by virtue of association, scientists have inferred that there is some degree of specificity between the genotype of _P_. _syringae_ and the plants on which they are likely to reside. Paulin and Luisetti (1978) conducted a similar survey and reported that 79% of 134 _P_. _syringae_ strains that they tested were active as ice nuclei at -4 C.

Since the discovery that certain bacteria are active as ice nuclei, it has been recognized that not every cell in a given population is active as an ice nucleus at any given time and temperature, even though ice nucleation activity is an inherent genotypic property of a particular bacterial strain (Maki et al., 1974). The relative ice nucleation activity of an INA bacterial strain has been expressed in terms of its nucleation frequency--the ratio of active ice nuclei to viable cells (Lindow et al., 1982a). Strains of INA P. syringae are not all equally active in catalyzing supercooled water to form ice. Nucleation frequency can vary greatly among different INA strains cultured under the same set of laboratory conditions (Hirano et al., 1978, 1985). The median $\log_{10}$ nucleation frequency at -5 C for two collections of P. syringae were -4.37 for 154 strains isolated as plant pathogens and obtained from the NCPPB and -4.63 for 144 strains isolated from healthy leaves of different plant species grown in Wisconsin (Hirano et al., 1985). The median $\log_{10}$ nucleation frequency at -5 C for 82 P. syringae strains isolated from fruit tree orchards in Washington and Oregon was -4.0 (Gross et al., 1983). Thus, <1 in 10,000 cells of the average P. syringae strain is active as an ice nucleus at -5 C when grown on a given medium and tested in the laboratory. The conditions of bacterial growth can markedly affect nucleation frequencies (Lindow et al., 1982b). Thus, bacterial nucleation frequencies in nature may differ from those measured in the laboratory.

Erwinia herbicola. Although no systematic survey to determine the proportion of E. herbicola strains that are ice nucleation active has been conducted, it is generally thought that the ice nucleation phenotype is less frequently expressed within this bacterial species. Of 52 E.

herbicola strains tested by Paulin and Luisetti (1978), none were active at -4.0 C. Of 77 *E*. *herbicola* strains isolated in Wisconsin that were active at -10 C, 45% were also active at -5 C. The median $\log_{10}$ nucleation frequency at -5 C for these 42 strains was -5.93--only one active cell in a population of almost a million (Hirano et al., 1985).

Pseudomonas viridiflava, *Pseudomonas fluorescens*, and *Xanthomonas campestris* pv. *translucens*. The number of strains within these three bacterial species that have been tested for ice nucleation activity is relatively limited. Paulin and Luisetti (1978) found 8 (42%) of 19 strains of *P*. *viridiflava* that were active at -4.0 C. Seventeen isolates of *P*. *viridiflava* from diseased tomato leaves were reported to be ice nucleation active, although the assay temperature used was not specified (Jones et al., 1984). We have also isolated presumptive *P*. *viridiflava* from snap bean leaflets and find the proportion that are active at -5 C to be very low (i.e., one active strain of 34 tested). The proportion of *P*. *fluorescens* and *X*. *campestris* pv. *translucens* strains that are active as ice nuclei at temperatures where they are likely to be important as incitants of frost damage remains to be determined.

2. Among epiphytic bacteria in general.

The proportion of the total epiphytic bacterial populations that is likely to be ice nucleation active depends on several factors including, the epiphytic host plant species and cultivar, the geographic location of the plant species (or crop), the prevailing weather conditions, and probably interactions among these parameters. Populations of INA *P*. *syringae* frequently comprise greater than 10% of the total epiphytic bacteria on leaves of almond (Lindow, 1982), pear (Lindow, 1982), and oats (Hirano et al., 1981). In contrast, INA bacteria are usually present at less than 0.01% of the total bacteria associated with potato or navel orange leaves grown under California conditions (Lindow, 1982). In Greece, however, approximately 10% of the total bacterial population on orange leaves was identified as INA P. syringae (Constantinidou, 1985).

We have monitored total and INA P. syringae populations on cultivars of snap beans with known differences in field resistance to bacterial brown spot disease caused by P. syringae pv. syringae (Hirano et al., unpublished). At harvest time, the numbers of P. syringae differed by more than 1000-fold on the most susceptible compared to the most resistant cultivar, although the total epiphytic bacterial populations were similar on the two cultivars. P. syringae comprised 1 to 10% of the bacteria on leaflets of the susceptible cultivar, but less than 0.001% on the resistant cultivar.

For a single crop in a single geographic area, the proportion of the bacteria established on leaf surfaces that are ice nucleation active will likely fluctuate in response to fluctuating environmental conditions. For example, Lindow (1983) reported that total epiphytic bacterial populations were at approximately 10^6 colony forming units (CFU)/gram fresh weight of tissue from early March to May on tomato plants grown in California. However, the proportion that were ice nucleation active was less than 0.001% in early March and greater than 10% by late March.

C. Their habitats

1. The plant taxa upon which they live.

All five species of bacteria that are known to include strains that are ice nucleation active have been isolated from plants representing a diverse range of taxonomic genera and families. However, any listing of the plant taxa upon which INA bacteria are likely to be found (presented in Table 1) should be interpreted with some considerations in mind. (i) In the absence of an extensive and intensive sampling of all known plant species for the presence of each of the INA bacterial species, our knowledge of the plant taxa upon which INA bacteria have been found is naturally limited to those plant species which have been examined for their presence. To our knowledge, there has been only one deliberate survey of a large number of plants from several locations for INA bacteria per se (Lindow, et al., 1978a). The abundance, or lack thereof, of published reports describing the presence or absence of a given INA bacterial species on different plant species reflects, to some extent, the relative economic importance of those bacterial and plant species to agriculture. The _P_. _syringae_ group causes a number of important diseases, and thus, has been studied for many decades by plant pathologists. Hence, we know that many plant species will support pathogenic populations of _P_. _syringae_. On the other hand, _P_. _fluorescens_ is not known to be a foliar pathogen; _P_. _viridiflava_ has not been viewed as of major economic importance. Hence, the number of plant species (listed in Table 1) on which these two bacterial species have been found is limited, not necessarily because they do not colonize many types of plants, but more likely because no one has had a reason for looking for them there. (ii) The original descriptions of the five bacterial species of interest date back to the early 1900s. The literature, therefore, abounds with reports of isolations of, for example, pathogenic P. syringae, from many plant species. The ice nucleating property of P. syringae, however, was not discovered until the 1970's (Maki, et al. 1974). In many cases, we can only infer from our current knowledge that the P. syringae described in those reports were likely ice nucleation active. (iii) As discussed in a previous chapter, the taxonomy of the known INA bacterial species has undergone changes over the years. The absolute classification of an unknown bacterial species is not always readily achieved, as reflected in the taxonomy of INA members of the Enterobacteriaceae. Hence, in some cases, there may be an element of ambiguity in the range of habitats that can be assigned to a particular INA bacterial species because of the associated ambiguity in the taxonomy of that species. (iv) Negative reports are seldom published. Thus, although there may be few reports of the presence of certain INA bacterial species on plants, there are even fewer reports of their absence from particular plants.

The list of plant taxa presented in Table 1 is not inclusive. However, it illustrates the types of plants on which INA bacteria have been found. In the study of Lindow et al. (1978a), of 95 plant species sampled from five different geographic regions of the United States, 74 were found to harbor INA bacteria. Only the conifers, as a group, were likely not to support colonization of INA bacteria.

2. Other known and likely habitats. Soil and water.

Although soil and water have not been demonstrated to be favorable habitats for bacteria in the *P. syringae* group, they are primary habitats for bacteria in the *P. fluorescens* group (Starr et al., 1981; Palleroni, 1984). In spite of this, to our knowledge there are only two reports in the literature which describes the recovery of INA *P. fluorescens* from sources other than terrestrial plants. Maki and Willoughby (1978) isolated strains of *P. fluorescens* biovar V that were ice nucleation active from snow, from alpine lake water, and from stream water. INA *P. fluorescens* biovar V was also isolated from cultures of the marine dinoflagellate, *Heterocapsa niei* (Fall and Schnell, 1985). Although a search for INA *P. fluorescens* in sea water was not fruitful, INA *E. herbicola* (7 isolates of 1950) was recovered from the marine sample (Fall and Schnell, 1985).

D. What is known about the population densities and population dynamics of ice nucleation active bacteria.

1. Population densities

The population density of INA bacteria on leaves can not be described by a single, simple value. The range of densities of INA bacterial populations on plant species that harbor INA bacteria, is likely to be from none detected, where the limit of sensitivity of the detection method is generally on the order of 10-100 CFU per leaf or per gram of fresh weight, to $> 10^7$ CFU/leaf--the apparent carrying capacity of leaves of many plant species. We have found that at any given sampling time and on any given plant species, population sizes of INA bacteria can differ over 100-fold from one leaf to the next, in spite of the caution taken to harvest leaves of equivalent size (and therefore, presumably, equivalent age) and position in the plant canopy (Hirano et al., 1982). Although the mechanism(s) by which these variable population densities are generated are not known, what is known is that the distribution of bacterial population densities on a population of individual leaves within a given canopy can be described quantitatively by the lognormal frequency distribution. Hence, the best quantitative description of the population density of INA bacterial populations on a given crop at a given time includes the estimated median and variance of the lognormal distribution of bacterial population sizes. As will be discussed below (under population dynamics) fluctuations in the median and variance of INA bacterial population densities occur frequently throughout the growing season on annual plants. Host genotype, geographic location and associated cropping patterns, and weather influence the median and variance of INA bacterial densities at any given time.

2. Population dynamics: Seasonal trends.

Changes in population sizes of INA bacteria on a given plant species have generally been monitored by sampling leaves at weekly or biweekly frequencies for annual crops or at monthly intervals for perennial crops, such as fruit trees (Lindow et al., 1978a,b; Lindow, 1982; Gross et al., 1983). From these studies several generalizations can be made. For perennial fruit trees, including pear, almond, apple, cherry, and apricot, maximum population densities of INA bacteria are established on buds and flowers when weather conditions are cool and moist (i.e., spring months). Increases of INA bacterial populations on the order of 100- to 10,000-fold (i.e., from approximately 100 to greater than 10^4 CFU/gram fresh weight of tissue) occurred over a 4 to 6 week period following bud break on almond and pear leaves (Lindow, 1982). With the onset of warmer and drier conditions (i.e., summer months), INA bacterial populations decreased. Return of cool and moist conditions in autumn coincided with some increase in INA bacterial population sizes.

Annual plants in California responded similarly. Increases and decreases in INA bacterial population sizes responded to cool/wet and warm/dry weather conditions, respectively, on potato and tomato (Lindow, 1982). Under favorable weather conditions, over 1000-fold increase in population sizes of INA bacteria occurred on tomato leaves during the first 3 weeks following transplanting. In Wisconsin, population sizes of INA bacteria on several annual crop plants were quite low shortly after germination, increased as the plants grew, and either reached a peak and began to decline near the end of the growing season, or remained high until the plants were killed by frost (Lindow et al., 1978a).

3. Population dynamics: Daily changes.

Weekly and monthly sampling frequencies provide information on the long-term trends in INA bacterial population densities. However, these sampling frequencies are not adequate to examine the inherent dynamics of leaf-associated bacterial populations. To examine the population dynamics of *P. syringae* on snap bean leaflets in a way that would detect the rapid growth (or death) of which the organism is capable

(Young et al., 1977), we have monitored P. syringae population sizes on two time scales: (i) every two hours over a 26 hour period for randomly selected 26-hour periods in the growing season and (ii) every morning, five days a week throughout the growing season (approximately 6 to 7 weeks for snap beans). Ice nucleation active P. syringae is frequently the predominant bacterial species present on snap bean leaflets in Wisconsin. Our current understanding of the population dynamics of P. syringae has emerged from these experiments involving short-term sampling frequencies for population sizes of P. syringae coupled with continuous monitoring of the physical environment (Hirano and Upper, 1984, 1985, 1986). The dynamic nature of these populations is illustrated in the season-long profile in figure III-1. Population sizes of P. syringae can change rapidly. Population increases on the order of 155-fold, 400-fold and even up to 2300-fold occurred within 24-hour periods. On the assumptions that these increases were due to bacterial multiplication and that exponential growth occurred at a constant rate during each of the 24-hour periods, we estimated that generation times of P. syringae under field conditions on these three occasions were 3.5, 2.7, and 2.1 hours, respectively. However, rapid growth is the exception, not the rule. Fluctuations in population size of P. syringae on the order of 5-fold or less within 24 hours occurred much more frequently than changes on the order of 100-fold or more. However, only a single event in which populations increase 100- to 1000-fold is sufficient to increase the temperature at which the crop is at risk to frost injury by 0.5 to 0.75 C.

The environmental factor that appears to have the greatest effect on the population dynamics of P. syringae is rain. Figure III-2 illustrates changes in P. syringae population sizes from day to day for the same planting of snap beans for which median populations were illustrated in Figure III-1. The rapid increases in population sizes of P. syringae were frequently preceded by a rain event. In the absence of rain, population sizes of P. syringae tend to decrease (or at least not increase) even though fluid water in the form of dew may be present on the leaflets for several hours each night.

We can view the plant canopy of a particular crop as being composed of a population of huge numbers of individual leaf ecosystems ($>10^7$ per acre for snap beans). Each leaf may harbor a microbial community that differs from the next in terms of its genotypic composition and numbers of individuals present. These leaf ecosystems are variable and dynamic. Our studies have focused on a specific system: P. syringae on snap bean in Wisconsin. This organism, in this system, appears to respond to perturbations of the system by rain with rapid growth. Its life strategy, then, may be more population independent in nature, or "R" selected, then some other members of the leaf community. We need to determine whether our findings on the effect of the physical environment on short-term changes in population sizes of P. syringae are applicable not only to P. syringae on snap beans and in Wisconsin, but to other crops and on other locations before generalizations can be made.

4. Population dynamics: The role of dispersal

The role of dispersal in the population dynamics of this bacterium can be described - at least qualitatively. It is clear that INA bacteria are dispersed in space through the air, and between generations by seeds (cf. Hirano and Upper, 1983). Seed of all, or nearly all of the crops that are frequently infected by diseases caused by INA members of _P_. _syringae_ probably carry at least some level of infestation to the new crop, wherever it is planted. The extent to which the seed borne inoculum is important in establishing the eventual populations of INA bacteria probably depends on a very large number of other factors. The amount of halo blight of oats on young plants was positively correlated with the frequency with which oat seeds were infested with _P_. _syringae_ pv. _coronafaciens_ (Hirano et al., 1982b). On the other hand, the population sizes of INA _P_. _syringae_ that developed on small plantings of snap beans from the same (infested) seed lot were highly dependent on the density of commercial snap bean plantings in the areas in which they were planted (Lindemann et al., 1984). The most probable explanation for the latter observation is that bacteria are removed from leaf surfaces and dispersed by the wind. This occurs on most dry, sunny, windy days (Lindemann et al., 1982; Lindemann and Upper, 1985). Although the majority of the bacteria that are entrained in the wind are carried aloft, a relatively small proportion of the airborne bacteria are redeposited, usually very near their site of origin. In 1982, on an average sunny summer day, more than 10^4 bacteria were blown off of, and more that 1000 were deposited on the average bean leaflet

(Lindemann and Upper, 1985). Of these, about one percent were INA P. syringae. Since this sort of dispersal occurs on about half of the typical summer days in this area, and since most of the redeposition is probably very close to the site of origin, steep dispersal gradients can be created by aerosol dispersal. When populations of INA bacteria in the regions of these gradients are amplified by growth following rain, and when the population dependent infection of the host plants follows, steep disease gradients are observed. In the past, these disease gradients have frequently been interpreted as due to rainsplash dispersal of the pathogen, which lead to infection. The relative quantitative importance of rainsplash in the population biology of these organisms makes this interpretation quite unlikely, however (Hirano and Upper, unpublished; Lindemann and Upper, 1985; Constantinidou et al., 1986).

The potential for less intense, longer distance aerial dispersal of leaf associated INA bacteria exists. Aerosol dispersal may introduce small numbers of bacteria, possibly representing different genotypes from those established on the plants at the site of introduction, at substantial distances from the site of origin. In one instance, when bacteria were deposited within a canopy from aerosols, this distance may have been as much as 60 km (Lindemann and Upper, 1985). The cycle of airborne dispersal of these bacteria is probably completed by rain, which scrubs the atmosphere, and redelivers the bacteria to plant canopies (Constantinidou et al., 1986). The INA bacteria may actually participate in this process, by serving as the ice nuclei that initiate precipitation. Indeed, there may be a substantial selective advantage to

INA bacteria by improving their chances of escaping from a cloud and returning to their normal habitat - a leaf - by creating their own reentry vehicle, a raindrop. In chapter VI of this report, we discuss the importance of ice nuclei to precipitation processes. The entire thrust of this report is to attempt to assess the extent to which bacterial ice nuclei may influence these processes, and the extent to which precipitation may be influenced by artificially altering numbers of ice nuclei on crops for protection against frost damage. Our knowledge of the very rapid fluctuations in INA bacterial population sizes, and of aerosol dispersal are based on studies conducted in a single area on a few plant species. The extension of these findings to other geographic regions and other plants needs to be accomplished before they can be used, with confidence, as general models for the population biology of these organisms. If control of frost injury by any strategy designed to alter bacterial populations is to be pursued, a strategy based on an understanding of the quantitative population biology of INA bacteria, in general, is the most likely to succeed. Such an understanding is yet to be achieved.

E. Competitive interactions between INA and non-INA strains.

Competitive interactions between INA^+ and INA^- bacterial strains have been examined under controlled environmental conditions (i.e., in greenhouses or growth chambers) with test plants that lacked any of the bacterial epiflora present on plants grown in the field (Lindow et al., 1983a; Lindow, 1985; Lindemann and Suslow, 1985, 1986; Lindemann et al., 1985). Under these conditions, no evidence has been obtained which would suggest that the INA phenotype, or lack thereof, influences epiphytic colonization ability. What is apparent from these experiments is that 'he who gets there first is likely to be there last'. The time between application of the competitor (e.g., INA$^-$ strain) and the target organism (e.g., INA$^+$ strain) appears to have a significant effect on the proportion of the final population that is comprised of the target organism. The sum of population sizes of the two strains remains constant, only their proportions change. For example, Lindow et al. (1983a) reported that the potential INA$^-$ biocontrol agent, _E_. _herbicola_ M232A, was effective in attenuating growth of an INA$^+$ strain and hence, frost protection only when it was applied any time before and up to 12 hours after the application of the INA$^+$ strain under growth chamber conditions. Similar results have been found with the genetically engineered ice-minus mutants of _P_. _syringae_ and _P_. _fluorescens_ (Lindow, 1985; Lindemann and Suslow, 1985, 1986; Lindemann et al., 1985). Lindow has suggested that successful antagonists act by preemptive exclusion, that is, they prevent increases in population sizes of INA bacteria, rather than displacing already established populations (Lindow, 1985). Thus, he proposes that competition for limited resources rather than antibiosis may be sufficient to explain antagonism between the closely related INA$^-$ and INA$^+$ strains on the leaf surface. For those cases where biological control of frost injury was achieved by application of either naturally occurring ice-minus strains (Lindow, et al., 1983a,b) or chemically-induced ice-minus mutants under field conditions, the competitor was always applied at a time when population levels of ice-plus bacteria were low. Thus, similar to results obtained under controlled conditions, preemptive competition appeared to occur between the two phenotypes in the field.

It is difficult to assess the proportion of field trials in which INA^- bacteria have been successful in establishing large populations on leaves and/or in minimizing frost injury to the target plant since negative results are seldom published. Under natural field conditions, wild type INA bacteria and any applied ice-minus strains are but two components among many that may colonize a given leaf surface. This microbial community is subjected to diurnal and daily fluctuations in the physical environment, unlike the relatively constant environment provided for in laboratory competition studies. Until we have a better (or at least some) understanding of the interactions that are likely to occur within entire bacterial communities, and the influence these interactions are likely to have on interactions between individual bacterial species or genotypes within a species under different environmental conditions, it is difficult to predict the relative competitive ability of any ice-minus versus ice-plus bacterial strain. For example, the naturally-occurring non-ice nucleating strain of E. herbicola (M232A), originally isolated from corn leaves, showed potential as a biocontrol agent for frost injury when tested under controlled conditions and in the field (Lindow et al., 1983a,b). The successful field trials were conducted in 1976 in Wisconsin. In 1977, J. Lindemann (unpublished) retested M232A in the same field experimental area. No significant reduction in population sizes of INA bacteria or of frost injury was achieved. In 1978, the ice-minus M232A was applied to at least two plantings of corn (Hirano, unpublished). On some occasions, the applied bacterium was recovered from the treated corn plants, on others, it was not. Since bacteria have the potential to produce many generations of progeny in relatively short time intervals, there is ample opportunity for selections to occur within the growing season of the host plant. The factors (physical or biological) that influence the selection of genotypes that will be most successful under a given set of environmental conditions are to date, unknown.

F. Summary

In summary, five species of bacteria are known to be active as ice nuclei (*Pseudomonas syringae*, *P. viridiflava*, *P. fluorescens*, *Erwinia herbicola*, *Xanthomonas campestris* pathovar *translucens*). All are frequent residents on leaves of plants and, indeed, only the conifers as a group have been found not to carry measurable populations of INA bacteria. Among the five species, *P. syringae* appears to be the most efficient ice nucleus and probably the most important with regard to frost injury. The current (and rather limited) understanding of the population biology of INA bacteria is based primarily on studies on *P. syringae*. The population densities of *P. syringae* and the proportion of total epiphytic bacterial populations that may be composed of the bacterium are dependent on a number of interacting factors including host genotype, geographic location, and weather conditions. However, no intensive and extensive studies have been conducted to assess the quantitative influence of these various factors on populations of INA bacteria. Population densities of

P. syringae can change dramatically and rapidly (over 1000 fold in 24 hours) with time. Large population increases have usually been preceded by rain. Epiphytic bacteria are dispersed into the air as aerosols. INA bacteria are likely to be carried aloft during dry sunny days. They may be redeposited on plants in low numbers from aerosols as far as 60 km downwind from the source, or by rain.

CHAPTER IV. THE POTENTIAL MAGNITUDE OF THE PROBLEM: IF APPROPRIATE TECHNOLOGIES WERE AVAILABLE, HOW MUCH CROPLAND MIGHT BE TREATED TO PROTECT CROPS FROM FROST INJURY.

In this chapter we will discuss the size and proportion of the U.S. land area that may be treated to protect plants from frost injury. The land area on which all of the plants grow that are likely to support growth of INA bacteria will be considered as the total area likely to function as a potential source of airborne bacterial ice nuclei. The proportion of that area that is devoted to production of frost sensitive crops constitutes the largest area likely to be treated for protection of crops from frost by removing or inactivating bacterial ice nuclei. The actual land area that may be treated to protect crops from frost by decreasing numbers of INA bacteria will depend on a large number of factors.

A. What proportion of the area of the U.S. is covered by plants that are likely to harbor INA bacteria?

The only group of plants that has been found consistently to harbor very low, or non-detectable population sizes of INA bacteria is the conifers (Lindow et al., 1978a). Population sizes of INA bacteria have been found to be very low on other plants in some locations, or in some seasons, but most of the plants that have been examined appear to harbor at least some of these bacteria on occasion (cf. Lindow et al., 1978a). Thus, the only plants that we will exclude from our calculations as a group are the conifers. Table 2 lists the land area of each state, the area of coniferous forests, and the difference between the two. Only those coniferous forests that tend to occur as relatively pure stands were subtracted from the total land area; forest types that include mixtures of hardwoods and conifers were not. We will use the total area less the area of coniferous forests as our estimate of the total area available for production of plants that harbor INA bacteria. This would correspond to roughly 97.5% of the U.S. land area (excluding Alaska and Hawaii). This is an overestimate, in that it includes man-made structures such as buildings and highways, and natural areas such as mountain tops and lakes where plant densities may be rather sparse. The extent of overestimation due to the inclusion of these areas, however, is relatively small. A much more serious source of error is that all plants other than conifers are assumed likely (equal) producers of INA bacteria, regardless of species, location, or canopy density. This is an oversimplification. Population sizes of INA bacteria differ on different plant species. At any given location and time, these differences may be greater than two orders of magnitude. The assumption is necessary because likely INA bacterial population sizes have not been determined for the vast majority of plants. The differences between climates in different regions is not considered, in spite of the fact that population sizes of INA bacteria are known to increase substantially (more than two orders of magnitude in some cases) in response to rain. The assumption is necessary because we simply do not know much about the ecology of INA bacteria in many areas. Canopy density is neglected. The ratio of leaf area to land area varies between plant species, between plants of the same species of different ages (sizes) and with plant population density, as may be affected by climate or agricultural practice. Errors associated with plant canopy density may be smaller than those associated with plant species and climate, but still may introduce errors approaching one order of magnitude.

Thus, by this assumption (that all non-coniferous plants are equal sources of INA bacteria) we are considering arid regions of the western U.S. to be equivalent to rain-watered cropland of the midwest for production of these bacteria. Not only are the plant species and population densities different between these two areas, but so are the frequencies with which INA bacterial growth is likely to be stimulated by rain. Thus, our assumption that all of the U.S. land area that is not covered with coniferous forests is covered by plants that harbor INA bacteria may be acceptable qualitatively, but it is a likely source of substantial quantitative error. Research that examines the population biology of INA bacteria on many different kinds of plants in the several different climate zones of the country is necessary before this assumption can be refined substantially.

B. What proportion of the area where INA bacteria are likely to reside is in cropland?

Table 2 contains a listing of the amount of cropland in each state. Cropland probably includes all of the land on which any crops will be grown that might, potentially, be treated with procedures designed to remove, displace or inactivate INA bacteria. Thus, land suitable for production of frost sensitive crops constitutes about 25.6% of the area available for production of INA bacteria in the U.S. (24.9% of the total U.S. land area). Since many crops are not susceptible to the kind of injury that INA bacteria are likely to cause, it is not reasonable to assume that an area this size will be treated.

C. Which crops are susceptible to frost injury of a type that might be controlled by decreasing the population sizes of INA bacteria on them? What proportion of the agricultural land area of the U.S. do these crops occupy?

Tables (3-6) contain listings of crops that are potential targets of technologies designed to protect crops against frost injury by decreasing the numbers of bacterial ice nuclei. Table 3 includes most of the extensively grown field crops that are likely to be damaged by frost. Table 4 includes major acreages of vegetables that are susceptible to frost injury. Table 5 lists acreages of frost sensitive deciduous fruit crops that are likely to be frost injured. The area planted to citrus fruits is presented in Table 6. The frost-sensitive crops listed in tables 3-6 constitute about 43% of U.S. cropland, or 10.8% of the total area of this country. Some crops that are grown on small acreage have been omitted. However, since many acres of the crops that have been included are grown under conditions where frost injury is extremely unlikely, barring nuclear winter or the like, these tables represent a substantial overestimate of the acreage likely to be treated.

A few comments on some of the assumptions that have gone into compilation of the tables. Some crops (e.g., cotton, sugar cane) have not been included since sufficient damage will be done by low temperatures above freezing that frost injury would amount to killing a physiologically impaired plant. Some winter annual crop plants have been excluded since more benefit may be gained from bacterial ice nucleation for winter survival than lost by spring frost. Winter wheat is the most extensively grown crop in this category in the U.S. The fact that we do not expect large losses to frost damage to this crop in the U.S. additionally influenced our decision to omit it. A number of crops that may be damaged by freezing, but normally are not damaged in the temperature range in which bacterial ice nucleation is important have also been excluded (cabbage). Although citrus crops are included, ice nucleation active bacteria may not be important to that crop in some regions, particularly in Florida. Population sizes of INA bacteria on citrus in Florida are too low to have an important effect on freezing of that crop in that location. In California and in Greece, however, INA bacteria may be important to frost injury to citrus. The many crops that have been omitted by virtue of their very minor acreage may indeed be severely plagued by frost injury. However, their omission will not affect the conclusions of this report. Some of these crops may have a very local effect on precipitation--on the scale of a single thunderstorm. Thus, some of the crops that have been omitted may have the possibility of modifying local precipitation.

D. How much of the area on which sensitive crops are grown is at sufficient risk to economically significant frost that treatment would be likely if available?

Since we do not have any way to estimate the extent to which the crops listed in tables 3-6 are likely to be treated, we will assume that all of the acreage will be treated. This is obviously an overestimate; a worst case of a worst case scenario. On a national scale, large acreages will not be treated due to the fact that some crops are produced in areas and in ways that do not constitute sufficient frost hazard to warrant expenditure of resources to protect them against frost. Other crops are produced for a small enough economic margin that growers may prefer taking their chances on frost injury to spending the money for avoiding frost injury. Some crops will not be treated because effective technologies may not be available for use on them. Indeed, this is clearly the largest factor preventing widespread use of this type of technology for frost prevention today and it will probably remain the major obstacle to its use in the near future.

Although equating the acreage of frost sensitive plants to acreage that is likely to be treated is absurd on a national scale, on a local, or even regional basis, this may not be a serious overestimate at all. Production of many crops is substantially localized in this country. It is quite conceivable that a technology could become used extensively in any particular area. Thus, the assumption that all of the frost sensitive crops are likely to be treated could be very reasonable for areas the size of counties, or even large areas of entire states. These are large enough areas to be of real consequence for modification of precipitation (see chapters VI and VII). The percentage of the total area available for production of INA bacteria (table 2) that may be attributed to frost sensitive crops (tables 3-6) is shown for each state in table 7. These percentages range from 0.04% for Nevada to 61% for Iowa. Thus, although no more than about 11% of the lower 48 states may be treated, many areas the size of counties may be candidates for treatment of nearly all of their vegetation. Note the impact of a few crops on the states with large acres of "frost sensitive" crops. Iowa and Illinois head the list because of large acreages of corn and soybeans. Other areas would have substantially larger "frost sensitive areas" if winter wheat, peanuts, or cotton were included. Our overall conclusions would not be affected but different areas would appear to have increased risks.

Many crops require sufficiently short periods to grow that they can be planted at times and in locations where the probability of frost injury is insufficient to warrant either worry or monetary expenditure for its avoidance. However, even these crops are frequently planted at times, or in places where there is a substantial frost hazard. This is usually done by growers who are willing to accept the added risk of a possible frost in order to attempt to achieve the additional economic gain of producing the crop at a particular time. Very early or very late season prices for vegetable crops may be sufficiently higher than mid-season prices that growers may attempt to take advantage of them in spite of risks of frost losses. Corn hybrids that require longer growing seasons and are at risk to fall frost injury may be planted to take advantage of their greater yield potentials than earlier maturing hybrids. Thus, some crops may be raised under marginal conditions. But only marginal. Growers are unlikely to plant crops at times or in places where the odds do not favor success. Thus, the difference between complete loss and successful harvest may frequently be as little as 0.5 C. If effective technologies become available to decrease the risk of frost injury by decreasing the amount of bacterial ice nucleation on crop plants, individual growers will decide if the cost is worth the perceived decrease in risk. The extent to which they do this will depend on many economic factors, the experience of their neighbors, and the availability of appropriate technologies.

The extent to which farmers in the lower 48 states may benefit from frost protection can be illustrated by comparing probabilities of occurrence of damaging frost without protection to those if effective protection is available. If we assume that technologies capable of decreasing the temperature at which frost injury is likely to occur from -2.2 C to -4.4 C were available, what would be the change in the probability that a damaging frost would occur? On days on which the normal probability of the last spring frost at -2.2 C is 50%, that probability for a -4.4 C frost is below 20% at 141 out of 180 stations spread across the U.S. (NOAA, 1975). Similarly, for the first fall frost the 50% probability of a -2.2 frost is reduced to below 20% at 133 out of 180 stations. A reduction in temperature at which damage will occur from -2.2 C to -4.4 C corresponds to a reduction in probability of damaging frost from 50% to an average value of 16% nationwide, or below 25% at over 90% of the stations for both spring and fall frosts. There are a few states where the reductions are notably less that the average:

Kansas and Louisiana average 22% in spring, and Missouri and Kansas average 22% in the fall. In states along the northeastern coast, the decrease in temperature at which damage occurs from -2.2 C to -4.4 C corresponds to a decrease in probability of damage from 50% to less than 10% in both spring and fall. These reductions in frost probability ensure that the temptation to use technologies to protect crops from frost by interfering with bacterial ice nucleation will be presented to virtually all of the nations farmers.

E. Summary.

Plants that are likely to support growth of INA bacteria may occupy as much as 1,855 million acres, or about 97.5% of the area of the lower 48 states of the U.S. Cropland is about 474 million acres, or 24.9% of the area of the same states. Frost sensitive crops are grown on over 206 million acres, or 10.8% of the same area. Regional differences in acreage planted to frost sensitive crops are very large, ranging from about 0.04% of Nevada to 61% of Iowa. Although it is not likely that all of the land treated to frost sensitive crops will be treated to remove bacterial ice nuclei nationwide, on a regional or local basis, treatment of all or most of the land devoted to production of these crops seems more likely. The impact of reduction of the temperature at which crops are damaged by frost from -2.2 C to -4.4 C can be illustrated by comparing the probabilities of frost events at these two temperatures. If we take the day on which there is a 50% probability of frost at -2.2C (both spring and fall) for our basis of comparison, the average probability of a -4.4C frost is only 16% for 180 stations nationwide.

Thus, most of the nation's producers of frost sensitive crops will find such technologies tempting to consider.

CHAPTER V. MODIFICATIONS OF POPULATIONS OF INA BACTERIA OR OF BACTERIAL ICE NUCLEI THAT MAY BE ATTEMPTED IN ORDER TO MITIGATE THE FROST HAZARD TO CROPS.

In this chapter we will consider the changes in sizes of INA bacterial populations necessary for effective frost protection. The four alternative technologies by which these changes might be achieved will be considered. For each of these possibilities, we will discuss the likelihood of implementation, the extent of modification of sizes of INA bacterial populations, the likely duration of treatment each season, and the impact on INA bacterial populations on vegetation other than those actually treated.

A. What will the effects on population sizes of INA bacteria be on a national, regional, or local basis?

If we assume that the greatest impact on climate will occur if the largest number of INA bacteria are removed from all of the plants covering the land area in the United States, then two possible worst case scenarios might be considered. In the first, which we might term the direct effect, we will consider the possible consequences of treatment of all of the acreage of the crops listed in tables 3-6 with the most effective method for modifying population sizes of INA bacteria. In this scenario we will assume that the acreages and percentages of land areas treated will be those shown in table 7. In the second possible scenario, which we will term the dispersal effect, we will consider the possible impact if GEMs (a) were effective in decreasing numbers of INA bacteria on crop plants, (b) spread effectively to nearby crops and non-crop areas, and (c) effectively colonized the plants to which they spread to the detriment of native INA bacteria. The extent to which this might occur will depend on many variables, notable among them, the unknown, relative mutual specificities of bacterial genotype for plant genotype. We will assume that the area affected will be double the total area of frost sensitive plants per state (but not more than 100% of the area in a state).

The principal sources of uncertainty associated with these two possible scenarios include the following. Potential sources of error associated with the direct effect scenario include the assumptions (introduced in chapter IV.A) that all of the frost sensitive crops will be treated, (a likely overestimate of the extent to which INA bacteria will be affected) and that all non-coniferous plants, nationwide, are equally likely sources of INA bacteria. At least in some regions, this may lead to a serious underestimation of the fraction of the total population of INA bacteria that would be affected by treatment. If, for example, irrigated crops in arid areas tend to sustain larger population sizes of INA bacteria than non-irrigated plants, and if the biomass of crop plants under irrigation is substantially greater than the non-irrigated wild plants on adjacent land, then the effects of treatments on total numbers of INA bacteria available for transport into the atmosphere may be substantially underestimated by equating the proportion of land area treated to the proportion of INA bacteria eliminated. One additional assumption imbedded in this scenario is that treatments that affect populations of INA bacteria in one location (field) have no effect on populations of these bacteria in other areas (e.g., an adjacent field or wild area). However, there is some evidence that cropping patterns, and hence patterns of INA bacterial populations as selected by specific crop plants, do affect populations of INA bacteria in adjacent areas (Lindemann et al., 1984). The "dispersal" scenario is an attempt to include the likelihood that bacteria can spread from one crop to adjacent (or more distant) areas. Potential sources of error associated with this scenario include all of those associated with the "direct effect" scenario, except that the area affected by spread is assumed to be about equal to the treated area. This assumption is completely arbitrary but does provide a hypothetical situation for purposes of discussion. The extent of spread of INA bacteria in a region will be very strongly dependent on the relative specificities of plant genotypes for bacterial genotypes. We are unaware of reliable quantitative data relating the relative mutual selection for plants and INA bacteria, yet the fact that different genotypes of *P. syringae* tend to be associated with different crop plants is well documented (cf. Hirano and Upper, 1983). For a strain of non-nucleating bacterium that was selected for its competitive ability on one crop plant species to successfully colonize another, it must successfully compete with the genotypes present on the second plant species. These native bacteria are probably present on the new plant as a result of many iterations of dispersal, growth, and competition in the environment of the new plant.

Thus, they should be expected to have the potential to be effective colonists on that plant. Even if the new introduction is successful in becoming established and reproducing on the new plant, its proportion of the population on that plant may be limited by competition with better adapted conspecifics. Thus, a more precise statement of the assumption regarding spread is that sufficient mutual plant-bacterial selection exists to limit the expansion of the population of a particular genotype through spread to about one doubling. We expect that this is a rather extreme scenario, since the degree of plant-bacterial specificity is probably sufficient to render epiphytic microbial communities highly robust to dilution by any single genotype. We expect that the actual extent of effect to lie somewhere between the "direct" and the "dispersal" scenarios, and probably much closer to the former. Note, however, that spread to other fields planted to the same crop as the one that is treated will be more likely. Thus, at least on a local or regional basis, spread will tend to have the effect of decreasing population sizes of INA bacteria present on a given crop relative to the proportion of the crop actually treated.

B. The potential approaches to decrease either population sizes or ice nucleation activities of INA bacteria and the likelihood of their utilization for frost protection.

Four potential approaches that are discussed in this section include:

Ice nucleation inhibitors

Bactericides

Antagonistic bacteria

Plant genetics

The challenge facing those who wish to design these technologies is that for each ten fold decrease in INA bacterial population size, the temperature at which ice nucleation occurs on a given leaf is decreased by about 0.25 C. Thus, to achieve as little as 0.5 C protection against frost injury, about a 100-fold reduction in bacterial population size must be achieved. Thus, the objective is to produce very large decreases in INA bacterial population sizes, or in numbers of bacterial ice nuclei. Technologies that produce only small changes in population sizes of INA bacteria are not likely to be acceptable in the market place.

1. Ice nucleation inhibitors.

A number of materials with the property of inhibiting bacterial ice nucleation without necessarily killing the cells have been identified (Lindow, 1982b, 1983; Lindow and Connell, 1984; Lindow et al., 1978c). For example, a urea-sodium carbonate mixture was tested as a potential ice nucleation inhibitor for protection of almond against frost injury (Lindow and Connell, 1984). Although other materials have shown promise in the field, many are highly phytotoxic. At the present time, ice nucleation inhibition appears to be the least likely of the potential means of decreasing the threat of bacterial ice nuclei on crop plants. Its principal advantage is its immediate action. Time is not required for bacteria to die (or not grow in the first place) and for ice nuclei to decay. This should provide a substantial advantage in the marketplace, but is also closely related to the major limitation of this technique. To achieve a reduction in ice nuclei sufficiently large to afford adequate protection with a single application of any material will require phenomenal coverage of the plant, especially if any INA bacteria reside inside substomatal cavities, or in other protected sites on leaf surfaces. Thus, it is our expectation that use of ice nucleation inhibitors will be limited to protection of only about 0.5 C on most crops. Frost protection by use of ice nucleation inhibitors may be feasible on those few crops that have relatively small numbers of INA bacteria, such as potato or citrus crops in some locations. Thus, it is our expectation at the present time that this approach will have limited use, or will be used to increase the efficacy of other methods. This opinion, of course, will be subject to change should a truly effective and affordable material and application technology become available. To our knowledge, no ice nucleation inhibitors are currently available, or in advanced stages of development.

Ice nucleation inhibitors would probably be the least likely to affect precipitation processes. If the materials used were not bactericidal, but only inactivated bacterial ice nuclei, then neither population sizes nor genetics of INA bacteria would likely be influenced. If they caused inactivation immediately after application, then the number of applications would be limited since growers would apply these materials only when frost was imminent. As new bacteria grew, new ice nuclei would be produced. Thus, the impact of application on climate, if there were any, would be limited in time and space to those periods and locations where frost was expected and where a severe freeze had not already killed crops. Thus, the climatic impact of use of such materials would be only local, or possibly regional, and brief.

2. Bactericides.

Application of chemicals, such as cupric hydroxide, streptomycin and oxytetracycline, that are lethal to bacterial cells have been successful in reducing INA bacterial population sizes on corn, beans, potatoes, squash, tomatoes, pear, almond, citrus, and avocado (Lindow, 1982b; Lindow and Connell, 1984; Lindow et al., 1978c). Reductions in sizes of INA bacterial populations on the order of at least 100-fold have been achieved. This level of reduction would correspond to a decrease in the temperature of the onset of frost injury of approximately 0.5 C. Disadvantages of this approach include the lack of availability of consistently effective materials and the amazing ability of INA bacterial populations to recover from the treatment. Repeated use of bactericides, such as streptomycin, frequently result in the build up of bacteria that are resistant to the applied chemical (cf. Dye et al., 1958; Moller et al., 1981; Schroth et al., 1979). There is the additional disadvantage that although certain chemicals (e.g., streptomycin) efficiently kill INA bacterial cells on contact in culture, these bacteria may retain their ability to nucleate supercooled water for a period of time (Lindow, 1982, 1983). Unlike ice nucleation inhibitors, bactericides will probably be applied well in advance of frost to affect a decrease in population sizes of INA bacteria. Multiple applications in a single season may be expected, as programs to manage populations of INA bacteria evolve. Thus, the effect on total bacterial ice nucleus production could be very substantial if bactericides were applied to all of the 204,706,142 acres of frost sensitive plants listed in Tables 3-6.

Bactericides may be of two general types. A broad spectrum material that may be used to control many bacterial diseases, caused by different bacteria on a wide range of plant species is generally viewed as the most likely to be developable. The market size for such a product would obviously be more attractive than that for a material with a very narrow specificity, given equal, or even adequate efficacy. However, when the very great capacity for multiplication of epiphytic bacteria is considered, the wisdom of using such a broad spectrum material becomes questionable. Killing all of the bacteria present on a leaf may only have the effect of creating an environment in which bacteria well adapted to colonize a disturbed site will flourish. Thus, it is entirely possible that broad spectrum bactericides of this type will be effective for relatively brief periods, after which population sizes of INA bacteria may actually be increased. Similar situations have occurred when broad spectrum insecticides have been used to control many insect pests. If natural competitors or predators were adversely affected, populations of the target insect may have actually increased.

Relatively narrow spectrum bactericides, if they can be found, may provide more stable control. We might imagine that such materials might only affect *P. syringae*, for example, without destroying any of its natural competitors or antagonists on a leaf surface. Treatment of large acreages with materials of this type may provide substantial, sustained attenuation of production of INA bacteria. Since materials of this type may be highly effective, it is entirely possible that the greatest potential direct effect on INA bacterial population sizes due to application of something to plants may be achievable in this way. It is likely that materials of this type will affect population sizes of INA bacteria on plants near to, as well as within treated fields. The ratios of INA to non-INA bacteria (of different species) available for dispersal would be substantially altered by materials of this type. To the extent that immigration of INA bacteria to adjacent plants influences the population dynamics of these bacteria on nearby crops, the overall population size of INA bacteria in a region might be substantially altered. If the bacteria present on treated plants interact competitively with INA bacteria, spread of these bacteria to nearby plants may also tend to decrease population sizes of INA bacteria beyond the area actually treated.

Since narrow spectrum biocides are likely to have rather specific sites of action, they are much more likely to select for resistant genotypes within a pest population (in this case, INA bacteria). Thus, this type of bactericide may also select for genetically different populations of _P. syringae_, and other INA bacteria. If these bacteria have altered, or no ice nucleation activity, they may also affect the numbers of bacterial ice nuclei on plants to which they spread. Thus, in some cases, the "dispersal scenario" needs to be considered for bactericidal, as well as microbiological treatments.

At the present time, all of the discussion of bactericides as frost protection agents is highly speculative. One material, cupric hydroxide (Kocide, Kocide Chemical Co.) already has EPA registration for use as a frost protectant. Although this material has only limited efficacy and is phytotoxic to many plants, including many grasses, growers are using it for the purpose of frost protection. Hence, the importance of assessing the potential impact of the technology of controlling frost injury by decreasing INA bacterial population sizes is emphasized by its presence, and the possibility that the next entry into the market may be both more effective and more generally utilizable.

In spite of the large number of acres potentially available for treatment with bactericide for frost protection, the general perception in the agricultural chemical industry seems to be that the potential market size is too small to warrant the cost of development of these materials. Thus, the potential for bactericidal protection against frost injury is unlikely to be realized in the short run. Once an effective material becomes available, and the industrial perception of market size is influenced by growers' enthusiasm to "outsmart old Jack Frost" this picture may change very readily. If effective, narrow range, non-phytotoxic bactericides are developed, we expect them to be used quite broadly for reduction of the frost hazard, at least on high value crops. It is not inconceivable that such a material may find use on nearly all deciduous tree fruits, citrus in California, large fractions of the vegetables produced in spring, fall and winter, and even on some field crops, such as corn and soybeans in years in which the price is favorable and the weather threatening.

3. Antagonistic bacteria.

The prospect for biological protection against frost injury by addition of selected microorganisms, particularly GEMs, to the agroecosystem presents an ample opportunity for speculation. In trials to date, competing microorganisms have been about as effective as the best bactericide treatments (cf. Lindow, 1982, 1983). In some cases they have been a little less consistent (cf. chapter III.E). Nonetheless, it is clear that at least some degree of alteration of the population sizes of INA bacteria can be achieved by addition of non-INA bacteria to plants, and that some measurable degree of protection against frost injury can occur. The organisms that have been the most effective to date are non-ice nucleating bacteria that appear to be very closely related to the ice nucleating targets. Thus, non-ice nucleating strains of P. syringae, P. fluorescens, and E. herbicola have been evaluated as likely candidates for biological protection against frost injury. The obvious extension of the search for organisms as similar as possible to wild-type INA bacteria has led to the use of recombinant DNA technology. The approach taken by Lindow (1985) and scientists at Advanced Genetic Sciences has involved selection of highly competitive INA strains and specific removal of the ice gene phenotype by site-directed deletion mutagenesis. The resulting ice-minus mutant should behave identically to its INA parent in all functions that are not influenced by the presence of the ice gene. If we assume that the ice gene functions only in ice nucleation and that it bestows neither competitive advantage nor penalty at temperatures above freezing, then both parental and mutant strains should compete for the resources available on leaf surfaces as if they were completely identical. Laboratory experiments with GEMs to date would seem to support the validity of these assumptions (Lindow, 1985;

Lindemann and Suslow, 1986). The relative sizes of the populations of the competitor and its INA parent are heavily dependent on the relative proportions of the two that are applied, and the relative timing of application. The total number of bacteria is not affected, just the fraction of the population represented by each of the two strains. If the available resources are already occupied by one of the two, the other cannot become established. Thus, the type of competition that is occurring has been termed preemptive exclusion, or competitive exclusion (Lindow, 1985). The action of such antagonistic bacteria, then, would be comparable to the most specific of bactericides.

What are the prospects for commercial use of bacteria, particularly GEMs for protection against frost injury? The basic problem remains unchanged. About 10^4-fold decrease in INA bacterial population sizes must be achieved per 1 C protection. How effective are GEMs or other bacteria likely to be in achieving a level of protection that will convince growers that they are economically worthwhile? In the field experiments to date, done with naturally occurring bacteria selected for their ability to compete with INA bacteria, successful protection has frequently been achieved if the competitor has been applied before the population sizes of the INA bacteria have become large (Lindow, 1982, 1983; Lindow and Connell, 1985). Decreases in INA bacterial population sizes have been on the order of 100-fold, relative to untreated control plants, and have persisted, provided that the weather conditions have remained relatively non-conducive for growth of INA bacteria. To our knowledge, there are no reports of success with this procedure if the competitors were applied after moderate to large population sizes of the wild-type INA bacteria had been established, or under conditions particularly conducive to growth of _P_. _syringae_. All of these observations are consistent with a mechanism of action for these organisms akin to competitive exclusion.

The persistent question remains: Are antagonistic bacteria sufficiently effective to gain commercial acceptance? Is a 100-fold decrease in population sizes of INA bacteria a level of control that is likely to be economically viable, or can the level of exclusion of INA bacteria be elevated, reliably, to a level that is? One of the major attractions of GEMs is the possibility of improving the competitive ability of an organism by adding specific genes to it. The problem with this approach is that we do not know what resources limit population sizes of _P_. _syringae_ and other INA bacteria on plants. Thus, the selection of genes to insert into epiphytic bacteria to improve their competitive ability on leaves would not have a rational basis. Although improvements of this sort may eventually be forthcoming, they must be preceded by a great deal of research on the ecology of epiphytic bacteria. We not only need to know what resources limit populations of these organisms, but also the mechanism of the limitation before we can intelligently add genes to alter competitive ability. Relatively little effort in microbial ecology has been devoted to population biology in recent years. The sampling and modeling efforts necessary for examination of the leaf-surface ecosystem are quite different from those used for what might be termed macroscopic organisms. If each individual leaf or leaflet is viewed as an entire ecosystem from the perspective of a bacterial community, then picking a leaf, grinding it up, and plating bacteria from the resulting suspension amounts to destructively sampling an entire ecosystem. Many replicate ecosystems must be sampled to adequately estimate the leaf to leaf variability among ecosystems. The numbers of such systems available to sample are huge - on the order of 10^5 to 10^7 per hectar. However, it is possible to identify and enumerate only a small fraction of the organisms present. Most of the systems that have been studied in the process of developing models of population biology and diversity were not sampled destructively, nor were a nearly endless supply of replicate systems available to sample. Indeed, non-destructive, and nearly complete censuses were often possible, but the number of systems examined was usually very small. Thus, many of the techniques and models used by ecologists who study birds, higher plants, or even insects are not directly applicable to a study of the microbial ecology of the phyllosphere. Methods and models more applicable to microbial-leaf systems need to be developed as the study of the ecology of these systems progresses. It may be quite some time before genetic alterations to improve competition of microorganisms on leaves are made that have any real, sound theoretical or empirical basis. Thus, for the short term, we will probably be limited to the type of bacterial competitors that are being proposed for testing by the Berkeley, and the Advanced Genetic Sciences groups.

Another advantage of using GEMs is their patentability. Indeed, this may be the major factor for choice of competitors for commercial use. If naturally occurring strains cannot be patented, but genetically engineered strains can, we expect that the GEM will be the choice of firms attempting to create a product. So, we assume that whatever bacteria are developed for protection against frost injury will be GEMs. The next factor to consider in their favor for commercialization is that GEMs are relatively non-toxic to animals, fish, plants, and Salmonella. GEMs constitute a huge question mark with regard to their impact on the environment, but the impact on the environment appears to be more likely to be of the type considered in this report than a mundane matter of acute toxicity. Also in their favor is the simple fact that there is commercial interest in developing them for this purpose! There still seems to be sufficient glamor in being the first (or among the first) to develop biorational pesticides by genetic engineering, that products of this type are likely to overcome the "there isn't a large enough market" stigma that seems to affect development of bactericides. Thus, although the efficacy of the bacterial competitors may not be particularly good, they may constitute the first generally applicable treatment on the market with at least a modest prospect for effective frost prevention. As such, they may be used on a rather large acreage. The scenario that they might be the most broadly applied biocide for frost prevention in the relatively near future seems to be rather likely. This scenario assumes, of course, that suitable GEMs are developed (this is now in progress), are effective in field trials (which will soon be initiated) and approved by the appropriate regulatory authorities for widespread use. For this reason, we consider it conceivable that GEMs of a type that will act by preemptive exclusion might possibly be applied to all of the acreages of crops in Tables 3-6 that are likely to be subjected to freezing temperatures.

Although GEMs may constitute the most likely short-term technology for use for frost prevention, they are not really likely to be applied to this large an acreage. Thus, this scenario in its extreme, is extremely unlikely. In addition to the reasons cited above, that is, marginal and unreliable efficacy due to interactions with both the weather and established populations of wild-type INA bacteria, there are other arguments that can be raised that point toward less than total reliance on this technology. Bacterial specificity with respect to plant species is probably the most important. It is very clear that there is some degree of specificity among strains of P. syringae for their ability to sustain themselves in large numbers on various plants. Thus, it will probably be necessary to select and develop specific GEMs for use in frost prevention on each crop. If there are additional selection pressures imposed by the plant bioclimate, as we suspect there probably are, then different strains may have to be developed for different regions, and perhaps for different cultivars. Clearly, this need will place both technological and economic restraints on the extent to which this technology is adapted for frost prevention.

4. Plant genetics.

The most promising alternative to date is selection of plant germplasm that avoids INA bacteria. Bean plants that tend to avoid high population sizes of P. syringae were selected in a program designed to select plants resistant to bacterial brown spot, caused by this bacterium (Daub and Hagedorn, 1981). We have shown that the size of the *P. syringae* populations among a population of bean leaves can differ by as much as 1000-fold between commercial cultivars, in replicated plots, and that this trait is readily heritable in bean (Upper et al., 1985). Plant genetic material that decreases the population sizes of INA bacteria even further appears to be available. If manipulation of INA bacterial population sizes by host plant genetics is generally applicable to crop plants, then cultivars of crops that are frequently damaged by frost will probably be developed that exclude sizeable populations of INA bacteria. When cultivars with this property are available, they will undoubtedly be the method of choice for avoiding INA bacteria for purposes of frost protection. From the point of view of the grower, it is probably the least expensive, most reliable strategy available.

Cultivar development, however, is a process that requires years, or even decades for completion. This may take less than a decade for annual plants for which there is an active seed market, and for which appropriate germplasm can be quickly identified, to a century or more for perennial plants in which regular breeding programs are not currently underway. Thus, we will not be able to rely on plant genetics for protection against frost injury for some time. The technology for identifying and screening germplasm is available. However, the extent to which this property is incorporated into new cultivars will depend upon the degree to which the seed companies perceive this property as of importance for sales, and the ease of identifying and using appropriate sources of germplasm. Even when cultivars are available that decrease the level of INA bacteria by 10^3 or more, providing nearly 1 C of protection against frost, growers may wish to use additional protective methods, for example, use of GEMs. Indeed, conditions where indigenous population sizes of INA bacteria are low is exactly where a strategy of preemptive exclusion may be the most effective.

The plants that select against INA bacteria genetically carry the same total bacterial population sizes as do plants that do not bear this trait. Thus, larger populations of bacteria other than INA bacteria are normally present on these plants. If these bacteria are competitive with, or antagonistic toward INA bacteria on leaves, then these bacteria may be expected to influence population sizes of INA bacteria on the leaves of nearby plants to which they spread. The normal populations of INA bacteria are not available to disperse from these plants and affect populations of INA bacteria on nearby plants. Just as the continued presence of large acreages of susceptible snap beans has increased the likelihood of detection of _P_. _syringae_ pv. _syringae_ pathogenic to bean on most plants in the bean growing area (Lindemann et al., 1984), so would replacing these beans with resistant plants probably decrease that probability. Thus, the effect of cultivars that select against INA bacteria may have an effect comparable to the "dispersal" scenario for GEMs.

C. Summary.

Since dispersal of epiphytic bacteria is expected, some degree of establishment and growth of these bacteria on the leaves or plants to which they spread is expected. Thus, the area on which population sizes of INA bacteria are affected may be somewhere between the actual area treated for frost protection and some larger area that might be viewed as the limit to which epiphytic bacterial populations on other plants are affected by spread of bacteria from the treated area. Two scenarios were proposed, the "direct" scenario, in which the effect of a given treatment is limited to the treated area, and the "dispersal" scenario, in which an area about twice the size of the treated area is affected due to bacterial spread. The choice of double the apparent area is arbitrary, and probably much larger than likely, due to selection of each plant species for the bacteria that grow upon it.

Each of four potential technologies was evaluated with respect to the likelihood that it will be implementable, and the duration and area of its effect. Ice nucleation inhibitors are likely to affect only the area to which they are applied, and only at times when frost is imminent. Thus, they are likely to affect the smallest number of bacterial ice nuclei. They are also the least likely to be developed for commercial use, largely for technical reasons. Bactericides, particularly those with a relatively narrow spectrum, should they be developed, were found to have an effect for a longer period, and probably to affect more than just the treated area. Effective bactericides will probably be utilizable on many, or most crops, and thus may be applied to a relatively large area, if they become available. Although one material is currently registered for use for frost protection, we do not expect to see any effective bactericides on the market in the near future. There currently is commercial activity directed toward development of genetically engineered microorganisms for frost protection by competitive exclusion of INA bacteria. GEMs will probably spread beyond the treated area to some degree, and may provide adequate frost protection on a number of crops. They will probably have to be applied long before frost, several months in some cases. The area to which GEMs are applied may be limited by plant - bacterial specificity. Plant genetics can be used to develop cultivars of crops that select against INA bacteria. Cultivars of this type may provide the most reliable and least expensive protection against frost injury of all of the technologies. The area planted to the resistant cultivar will be affected season-long. Substantially more than just the planted area may be affected. Implementation of this technology will be slow, and limited with respect to crop.

With current data, we are not able to determine which of the possible technologies is likely to have the greatest impact on total numbers of bacterial ice nuclei, although ice nucleation inhibitors will probably have the least effect.

CHAPTER VI. WHAT ARE THE POTENTIAL CLIMATOLOGICAL IMPACTS OF LARGE SCALE AGRICULTURAL APPLICATIONS OF VARIOUS TECHNOLOGIES DESIGNED TO DESTROY OR REPLACE INA BACTERIA ON CROP PLANTS.

Particulate matter is the second major constituent of the atmosphere in addition to gases. Particulates (aerosols) influence the properties and processes of the atmosphere in a large number of different ways. The most important ones being the transmission of radiation, the chemical cycles and reactions of both the gaseous and aerosol components and the nucleation of cloud droplets and of ice crystals. Aerosols importantly influence the global climate, the formation of clouds and of precipitation, the chemical composition of rain, visibility, human health, dry deposition, atmospheric electricity, and many other characteristics of the atmosphere.

Bacteria, when lifted into the air from the ground or from plant surfaces exert some influence in all of the areas mentioned for aerosols in general, but, of course, they represent only a minute fraction of the total atmospheric aerosol content, so that their influence in most regards is not readily identifiable and can be expected to be miniscule.

In this report, only the possible impacts of changes in populations of ice nucleating bacteria will be discussed, and so only the role of bacteria as atmospheric ice nuclei will be considered and other atmospheric influences of bacteria are assumed to remain unchanged. At least to a first order of approximation this assumption is not difficult to justify. Whatever changes in the impact on radiation, on atmospheric chemical cycles or on condensation of cloud droplets would result, these changes would be secondary as they would arise from altered concentrations of the bacteria in the atmosphere, which in turn would result from changed transport of the bacteria due to their decreased ice-nucleating ability. The radiative, chemical and condensation-nucleation activities of individual bacterial cells are taken to remain unaltered by the deletion of ice-nucleation activity.

A. The importance of ice nuclei to precipitation processes.

There are two basic processes in the atmosphere which convert colloidally stable clouds into precipitation (the latter being defined as consisting of liquid or solid hydrometeors of appreciable fall velocities). The process of collision-coalescence, or "warm rain process", leads to the growth of hydrometeors by the gradual agglomeration of more and more cloud droplets into drizzle and then into rain drops, all in the liquid phase. The "ice-phase process" involves the growth of ice crystals from the vapor, and the subsequent accumulation, or riming, of supercooled cloud droplets to generate snow, graupel or hail. These may arrive at the ground as ice particles or may melt during their fall through air warmer than 0 C.

The relative contributions of these two processes of precipitation generation vary with geographical location, with meteorological situation and with cloud type. The fraction of annual or seasonal precipitation which develops by one process or the other can be stated only for a few areas in the United States. Most clearcut is the evidence for the overwhelming predominance of the ice phase process in the High Plains region, both in the winter and the summer seasons. The warm rain process is clearly dominant only in those sparse summer rains of the southern and south-eastern states which develop from shallow clouds. For all other areas and seasons there is an unknown sharing of the precipitation production between the two processes. One can only make the rough statement that the ice phase process is the dominant contributor to precipitation production over the U.S. as a whole. This is the generally accepted conclusion for mid-latitude climates.

The second part in the assessment of the role of ice nuclei involves an analysis of the details of the ice phase process. Here, again, there are formidable difficulties in generalizing on the basis of the meager research results now available. The major dilemma arises from the variety of avenues by which ice particles can originate in the atmosphere. The main distinction is between primary and secondary origins. The first one refers to the nucleation of ice from either the vapor or liquid phases, the second to ice germs originating from ice-ice or ice-water particle interactions. Furthermore, the growth from tiny ice germs to precipitation elements can proceed along a variety of simple or complex sequences, combining true crystal growth from the vapor with accretion of cloud droplets, while moving along trajectories of up to many hours in duration. The sketchy knowledge now available about how these elements of the processes combine in any given situation makes the question of the importance of ice nuclei a very controversial one. It can be stated from first principles that the transition from vapor or liquid to solid water must be initiated by an ice nucleus. Thus, one could make the generalization that in spite of the other complexities, the ice nuclei are a crucial part of the precipitation production process, and because of the general predominance of this process they are critical to the formation of a very large fraction of the precipitation of this country. On the other hand, one could argue that the existence of various avenues of ice generation, and the possibility of contribution by the warm rain process, plus the tendency for compensatory effects in natural systems of many degrees of freedom, reduce the role of ice nuclei to practically negligible levels. Neither physical, nor numerical experiments have so far given a fully credible basis on which to judge where reality lies between the two extreme positions.

Direct measurements of ice nucleus concentrations are not generally given a great deal of credence, mostly because of known short-comings of the measuring devices. Ice nuclei may act by deposition, condensation-freezing, contact-freezing, immersion-freezing and other intermediate processes. This complexity has not yet been adequately accounted for in the design of ice nucleus counters, hence existing instruments give only partial and indeterminate results. In fact, attempts to measure atmospheric ice nucleus concentrations have dwindled over the last ten years to practical inactivity. There are, at the moment, no accepted techniques, no measurement networks, no new data forthcoming. The examination of available data, with the caveats already mentioned, leads to some initial generalizations: (i) The concentrations of atmospheric ice nuclei increase exponentially with decreasing temperature (or increasing supersaturation with respect to ice), (ii)

Using a single technique of measurement, the concentrations of atmospheric ice nuclei exhibit, in the mean, about an order of magnitude variation with geographical location across the globe, and (iii) The temporal variation at a given site is 1 to 2 orders of magnitude, with occasional fluctuations of even greater magnitude. Figure VI-1 illustrates the first two of these points, and Fig. VI-2 is an example demonstrating the third. It must be emphasized that very few direct measurements of the airborne concentrations of atmospheric ice nuclei have ever been made at temperatures of -5 C or warmer, because of instrument limitations; detectable concentrations were found in a few instances by Schnell (1979). Fairly extensive data for the temperature region near -5 C are available for nuclei carried in precipitation (Vali 1971 and 1978). This temperature range is just where bacterial ice nuclei and their derivatives are likely to contribute most importantly to the populations of atmospheric nuclei. In view of the complexities just described, it is evident that the role of ice nuclei in forming precipitation can not be determined by attempting to establish correlations between the concentrations of ice nuclei and precipitation amounts or rates.

The experience accumulated with cloud seeding experiments, using artificial ice nuclei, is of some relevance to the question on hand. In these experiments the concentrations of natural ice nuclei are augmented in order to influence the precipitation process, the increases in ice nuclei concentrations being usually quite massive. Because this is such a large perturbation of the natural conditions, the relevance of conclusions regarding correlations of ice nuclei with precipitation must be considered with caution. Yet, in view of the paucity of other types of evidence, the large body of literature on cloud seeding studies is one of the better bases on which to examine the question. The evidence now available for the effectiveness of ice nuclei in altering cloud evolution is of two kinds: statistical and physical. The statistical evidence is the most definitive in demonstrating increases of additional precipitation at the ground. The two most widely recognized examples are the Israeli rain enhancement project and the winter snow augmentation tests in the Colorado mountains. The observational and modeling studies also show that, given the right circumstances, ice nuclei seeding can lead to increased numbers of precipitation elements in the clouds. However, these studies revealed that the conditions under which the induced changes are appreciable are rather restrictive. Many clouds have been shown to be naturally efficient in converting cloud water to precipitation. The literature on these topics is very extensive. A comprehensive survey of the status of weather modification was conducted in 1978 by the Weather Modification Advisory Board for the Secretary of Commerce (WMAB Report, 1978). This report contains material on the scientific bases and on the results of cloud seeding experiments.

Some further results, which appeared since 1978 are worth examining. The positive results of the Israeli experiment received further confirmation (Gagin, 1986). The experiments conducted in Florida over the period 1978 to 1980 also showed positive results, but not with convincing statistical significance (Woodley et al., 1983; Gagin et al., 1986). The physical outcome of seeding was clearly demonstrated in Montana (Cooper and Lawson, 1984). These results are the most clearcut ones as far as establishing a positive cause-and-effect relationship between seeding and the development of increased numbers of precipitation-sized ice particles. A broad-based experiment in Spain showed that the major rain producing clouds in the interior of the peninsula were naturally efficient (Vali et al.,1986). In some model calculations positive results of seeding have been shown for both convective and for stratiform clouds (Hsie et al., 1980; Orville et al., 1984,). On the other hand, the efficiency of clouds was found to decrease with increased ice nucleus concentrations in some other calculations (Murray and Koenig, 1976) and effects of both sign were found in the simulations of the dynamic stimulation of clouds via ice nucleus seeding (Levy and Cotton, 1984). Overall, one could say that recent results give cause for decreased optimism about the productivity of cloud seeding, mainly as a consequence of finding relatively high natural precipitation efficiency more widespread than was once thought.

The simplest, intuitive explanation for efficient precipitation production is that because of the existence of alternative processes for forming precipitation-sized hydrometeors, including alternatives for ice particle origins, there are no limiting steps which could exert a controlling influence on the evolution of precipitation. Ice nuclei were traditionally considered to hold such a position. On the other hand, it is also well established that cloud systems which are clearly inefficient (say, <50%) in converting the total condensate into precipitation do exist, as do clouds which lack ice particles even at temperatures of -20 C or colder. The point is that these situations appear to occur in clouds which have the potential to develop only small amounts of precipitation anyway. Special circumstances, like orographic forcing of the rate of condensation, and narrow ranges of convective cloud conditions are the limited situations where the potential for increasing precipitation by seeding are now thought to exist.

For the question on hand, the deduction one would tend to draw from the findings just summarized is that if relatively high natural efficiency of precipitation production develops in a wide variety of clouds in spite of presumed variations in ice nucleus concentrations, the role of ice nuclei would have to be judged minor. Arguing that atmospheric ice nuclei may be ubiquitous enough to ensure efficient precipitation initiation by themselves is inconsistent with the existence of supercooled, non-precipitating clouds. A further line of evidence countering the lack of importance of ice nuclei is the finding of copious numbers of freezing nuclei in summer rain and hail (Vali, 1971, 1978) and the correlation between the presence of these nuclei and their availability form the soil (Vali and Schnell, 1976). In contrast, snow, and also rain from areas with soils of poor nucleating ability, contain few active freezing nuclei. While it would be tempting to put a great deal of weight on this evidence, caution must be applied since the passive scavenging of the nuclei by the rain can not be completely ruled out at this point.

The upshot of having to consider such a variety of conflicting and relatively frail facts is that one can not make a more clearcut general statement on the role of ice nuclei in precipitation formation than the following:

> Ice nuclei constitute one of several factors determining the efficiency of clouds in producing precipitation. The weight of their importance varies from none to dominant, depending on cloud type and on other meteorological conditions. The role of the nuclei can be assessed reasonably well by direct observations and can be deduced, to some extent, from the meteorological characteristics of given regions.

This general statement can be made more useful by considering three factors: the natural efficiency of the clouds, the type of cloud system, and geographical location. Anticipating a more critical role for ice nuclei in clouds of low precipitation efficiency, one could focus attention on the cloud systems which are, say, less than 30% efficient. Here, again, there are hardly any data of direct applicability; a rough estimate for the fraction of precipitation which falls from cloud systems of <30% efficiency is 1/3 (for mid-latitude climates), so this fraction of clouds could be considered as importantly depending on the availability of ice nuclei for the initiation of precipitation.

Regarding cloud type, it appears justified to construct at least a partial list which goes from the precipitation type least likely to be dependent on ice nucleus concentrations to those most likely to be influenced by them:

Nimbostratus - steady light rain or snow

Cumulonimbus - thunderstorms

Cumulus congestus - showery rain

Altostratus and stratocumulus - drizzle

Shallow orographic clouds - mountain snow.

With regard to geographical differences, there appears to be an indication that the southernmost states, and especially the southeastern states may have cloud systems in which the ice-phase process is linked to the warm-rain process and in which secondary ice generation works efficiently, so that the role of ice nuclei is probably less in these clouds than in those of the remainder of the country.

Special consideration needs to be given to the situation with respect to hail. As it is frequently assumed in models of hailstorms and in conceptual descriptions of the hail process, one of the possible ways in which hail embryos can originate is the nucleation of ice at temperatures a few degrees below 0 C in the updrafts of the storms. The growth of hailstones is a sensitive function of where within the storm the initiation of embryos takes place (e.g. Knight and Squires, 1982). There is no simple generalization possible of the consequences of changing the input of ice nuclei to hailstorms. It seems, nonetheless, reasonable to anticipate that changes in the spectra of ice nuclei entering certain storms may produce more radical changes in the development of hail than in the total precipitation produced by the storms.

In summary:

> While there are several lines of refinements which, conceptually, could give better definitions of the role of ice nuclei in the formation of precipitation, i.e. differentiation by cloud type, precipitation efficiency and geographical location, the necessary quantitative information is not available at this time. Hence, it is evident that wide open statements like those of the preceding paragraphs can only be used with considerable judgement. On another level, the lack of definiteness in an area of such great importance is a clear indication that strong research efforts will be needed in the future.

B. The origins of ice nuclei in the atmosphere.

The concentrations of ice nuclei in the atmosphere are on the order of 1 to 10^4 m^{-3}; whereas the total particulate content of the air ranges from 10^8 to 10^{11} m^{-3}, i.e. ice nuclei constitute a miniscule fraction of the total aerosol population of the atmosphere. Consequently, their identification and analysis is extremely difficult. The major source of information on the composition and possible origins of the ice nuclei come from correlations of ice nucleus concentrations with air mass origins. In addition, micro-analyses of the particles found at the centers of snow crystals have been used, with the assumption that those particles were ice nuclei. These approaches lead to the identification of clay minerals as the most likely constituents of ice nuclei - a conclusion intuitively acceptable in view of the wide availability of clay minerals at the surface of the earth. However, reservations are in order in view of the indirect nature of the evidence and because of the relatively poor nucleation activity of clays when tested in the laboratory, as discussed below. Also, it must be recognized that aerosol particles are almost always composed of a mixture of substances so, while the clay component may be the most readily detectable due to its large mass, the nucleating activity may well reside with some minor component of the aerosol (direct evidence for this possibility was given by Schnell (1977).

A third avenue of approaching the question is the examination of the nucleating activities of various substances which have appreciable abundances at the earth's surface. These laboratory studies gave some support to the importance of clay minerals, but also showed, over the last ten years, that organic substances of biological origin may play a special role. The most clearly-identified biogenic ice nucleus so far is the bacterium *P. syringae* which is wide-spread enough to be considered a major potential contributor to the ice nucleus populations of the atmosphere. There are further bacterial species having similar properties, but these have smaller abundances and are less active ice nucleators. The major characteristics of the bacterial ice nucleators are detailed in reviews by Hirano and Upper (1985) and by Lindow (1982a; 1983), and are also discussed in Chapter III of this report. The overall importance of the bacterial nucleants in the atmosphere is not yet clear. While there is evidence for the short-range transport of these bacteria, long-range transport has not yet been demonstrated, and neither has the retention of nucleating ability during atmospheric transport.

Another group of biogenic ice nuclei have been found in decayed plant material (Schnell and Vali, 1976), i.e. in soils of high organic content. These nuclei have been investigated to a much lesser extent than the bacterial nucleants. Their identity or atmospheric abundance are largely a matter of speculation at the moment. The correlation between the soil types and the ice-nucleus content of rain, by climatological regions, is perhaps the strongest argument now available for the importance of these nuclei. The shedding of nucleating material from bacterial cells (Phelps et al.,1986) may be a possible link between the bacterial cells as nucleators and the organic nuclei in soils. Such a link would greatly enhance the importance attached to the ice nucleating bacteria. Furthermore, one can hypothesize that the organic ice nuclei frequently become attached to clay particles in the soil, so that the evidence linking clay particles with atmospheric ice nuclei may be indirectly supporting the importance of organic (and probably bacterial) nuclei.

The observed capacity of bacterial and organic-soil nuclei to nucleate ice even at temperatures just a few degrees below 0 C, in contrast to clay particles which generally require -10 C or colder temperatures, puts a special emphasis on these nuclei. Their high activity is certain to make the role of the biogenic nuclei in the production of precipitation much greater then their overall numerical abundance would indicate. The basis for this statement is the advantage that ice particles originating at relatively warm temperatures have in achieving large sizes. There is evidence that the effluents from metal smelters are sources of ice nuclei. The specific nature of these nuclei has not been established. It is likely that metal oxides are involved. The overall national or global impacts of such sources are not known but are not suspected to be large.

Another approach to characterizing the sources of ice nuclei, albeit without gaining insight to their identity, is to examine the distribution of the nuclei in space and time. Reference to the general characteristics of this distribution has been made already in the preceding section. More complete reviews of the subject were given by Mossop (1963), Knight (1979) and Vali (1986). In rough terms - because of the uncertainties with all past measurement techniques - it may be stated that the lifetimes of atmospheric nuclei are at least as long as that of water vapor, on the order of a week, so that a relatively homogeneous background population of nuclei is established over most of the globe. Superimposed on this background concentration are smaller scale and variable magnitude local perturbations. The sources of these have been speculated to be streams of extraterrestrial particles, volcanoes, dust-storms from deserts, industrial pollution or local soil lifted by convective currents.

With respect to the bacterial ice nuclei, or their derivatives, there is no basis at present on which to judge the possible contribution they might make to the long-term average, or background concentrations of ice nuclei. Their importance on the local scale is intuitively much more likely, and the correlation between the nucleus content of precipitation and soil type is a fairly clear indication to that effect. There is no good basis from which to specify in further detail the relative contributions of bacterial ice nuclei, which reside primarily on the plants, vs. the soil nuclei. This will depend not only on the concentrations available in the two sources but also on the mechanisms of release by which the nuclei get lifted into the air. From the latter point of view, in areas of dense crops or vegetation, dominance by the bacterial ice nuclei could be expected. The main point concerning INA bacteria and biogenic nuclei in soils is that:

> While the origins of atmospheric ice nuclei must be considered to be mostly unknown, bacterial ice nuclei and their derivatives can be expected to make a significant contribution, especially to the populations of nuclei active at small supercoolings.

Further to examining the origins of ice nuclei and their physical activities in cloud systems, consideration needs to be given to the mechanisms of transport by which nuclei may enter the clouds.

Based on the evidence just discussed concerning the conceptual division of ice nucleus populations into background and local components, their entry into different cloud systems can be also be considered in two parts: the contribution of local sources, and the advected background concentrations.

The general guideline in this issue is that the greatest part of the aerosol content of a cloud originates along with the water vapor from which the cloud forms. A smaller portion of the aerosol may enter the cloud during the processes of mixing (entrainment) between the cloudy air and its environment. The validity of this statement is strongest during the active phases of cloud development. By the time the proportions might reverse, the cloud is most likely to be in a decaying phase in which precipitation production has come to a virtual end. Thus, the question is reduced to considering the source(s) of the air in which given cloud systems form. In general terms, stratiform cloud systems, which have small vertical velocities, form in air advected from long distances and hence can be considered to contain the background concentrations of nuclei. In contrast, convective clouds are dominated by vertical motion which has been shown to originate close to the ground, hence they will be strongly influenced by local sources.

The foregoing arguments lead to the conclusion that bacterial ice nuclei, and their derivatives residing in the soil, are most likely to have an impact on the development of precipitation in convective clouds. The entry of the bacterial or soil nuclei into these clouds is effectively ensured by the updrafts producing the cloud, and the concentrations of these nuclei can be high enough to exert a dominating influence on the total ice nucleus concentrations in the updrafts. As mentioned before, there is a possibility that in areas of dense crops or vegetation the INA bacteria may be the most important fraction of the total nucleus population. These expectations are subject to direct experimental verification, but the appropriate measurements have not yet been performed.

C. Expected effect on precipitation from the removal of INA bacteria.

It is evident from the foregoing sections that the physical link between ice nuclei residing on plant surfaces and precipitation development is far from simple, that it depends on the specifics of the precipitation type and that it can not be quantified with current knowledge. It follows, that predictions of the impacts of possible changes in the populations of INA bacteria will be subject to strong caveats.

Three factors need to be considered: (i) the magnitude of the potential change due to the removal of INA bacteria, (ii) the extent of the area over which precipitation might be affected, and (iii) the duration of the effect. These factors will be discussed, principally with convective clouds in mind, based on the arguments of the preceding section.

As pointed out in chapters II and V, in order to produce a useful reduction in frost damage the population sizes of INA bacteria would have to be reduced on the plants by at least a factor of 102. Making the simplifying assumption that there are no sources of atmospheric nuclei active at temperatures above, say, -6 C other than the direct or indirect bacterial sources, reductions of 100-fold or greater in the nucleus fluxes entering the air above the treated areas would produce a reduction in nucleus concentrations determined by the ratio of advection to local vertical flux. In the extreme case of no advection, the reduction in the air would be in direct proportion to the reduction on the plants. A

100-fold reduction in the concentration of airborne nuclei corresponds to a shift of 2 C to over 10 C in the temperatures of ice formation in clouds. (These figures are based on the observed slopes of ice nucleus spectra; the lower value is from the spectra found in precipitation samples at temperatures above -10 C, the larger value is based on measurements of airborne nucleus concentrations which usually show a 4 C to 10 C change for each order of magnitude change in concentrations.) The impacts of such changes in ice nucleus concentrations can be estimated to be comparable to what is accomplished by the seeding of clouds, as discussed in chapter VI.A. Considering reductions of 10 C or more,

in ice development would be tantamount to losing all competitive advantage of the ice nuclei to precipitation development, since by about -15C abundant ice development takes place anyway in most clouds.

The extent of the area possibly affected by reduced ice nucleus concentrations as a result of frost-prevention measures, has to be examined both in absolute terms and in relation to the sizes of precipitation systems. Considering the direct effect scenario, in which the removal of INA bacteria is confined to the crops actually treated, the simplest assumption is that the fraction of clouds influenced by the reduced availability of ice nuclei equals the fraction of total land area which is planted with frost-sensitive crops (i.e. likely to be treated). This fraction varies from near zero to over 60% on a statewide average basis (Table 8). The dispersal scenario (cf. Chapter V.A) doubles these percentages so that two states (Illinois and Iowa) would have their total land area devoid of INA bacteria and in eight states the fraction would be greater than 50%. As argued before, the most immediate effect is expected on convective clouds, with the effect roughly in proportion to the fraction of land treated. However, with increasing areal coverage of reduced concentrations of INA bacteria the consideration has to be broadened to include the "background" nucleus concentrations as well, and hence to extend the influence to stratiform clouds, even if not immediately over the area of concern but long distances downwind. Therefore, the fraction of precipitation influenced by the frost protection treatment is not in direct proportion to the fraction of area treated. For large areas with high percentage of frost-sensitive crops there is the possibility of additional effects, beyond the proportional effect on convective clouds. With the area of greatest coverage by frost-sensitive crops extending from North Dakota to Ohio, the large-scale effect on stratiform precipitation might be envisaged to encompass the mid-western and north-eastern states.

There is a second non-linear effect which needs to be considered. The preceding argument must be refined, especially for regions with low percentages of frost-sensitive crops, by comparing the patch sizes in which crops might be treated with the sizes of the inflow areas of convective clouds. These cloud updrafts are on the order of 1-10 km in diameter at cloud base altitudes. The simplest assumption is that the land surface from which the updraft's aerosol input is derived at any given instance is of the same magnitude. Using the above figures, the range of updraft areas is 250 to 25,000 acres, so that land areas of those sizes can be considered as source areas for clouds at a given time. The velocity of translation is quite variable for convective clouds, with zero to 10 m/s as the typical range. The time for a cloud to move one diameter, i.e. to completely change the source area at the ground, is between 10 minutes and 1 hour, which is roughly also the range of lifetimes for small to moderate-sized convective clouds. Hence, depending on the combination of parameters, land areas of several hundred to tens of thousands of acres will have the potential to exert a total influence on a particular convective cloud. Treated patches of only a few to hundred acres will influence clouds only partially, or only for a fraction of their lifetimes. The possibility can not be discarded that even a momentary influence can be determinant for the subsequent evolution of the cloud, but the risk is, clearly, getting reduced. The importance of patch sizes, or granularity, arises from the non-linear response of precipitation processes to inputs like ice nucleus concentrations. A given percentage change in input to all clouds will not have the same effect as a radical change in input to the same percentage of the clouds. The former may not produce noticeable effects, whereas the latter, possibly, induces a change in total precipitation of the same percentage as the influenced clouds.

The question of duration of influence, and its timing, can be viewed by assuming that the crop treatment changes the availability of nuclei only for the period during which treatment is necessary, i.e. during which threatening frosts occur or by assuming that not only the bacterial populations become altered, but that the alteration is not reversed within one growing season, and that treatment has been applied over several years in succession, so that the nuclei in the soil also become depleted (based on the working hypothesis that those nuclei are derivatives of the INA bacteria).

To assess what duration of change is implied by the first approach considered above, the difference between the dates of 50% freeze probabilities for 28 F and 24 F have been examined. (The warmer temperature is the natural threshold for moderate frost damage, while the lower temperature is the limit to which protection might be extended with treatment of INA^- bacteria.) From the climatological records for 1951-1973 (NOAA), the length of frost-free periods is found to differ for the two temperature thresholds by between 15 and 40 days for the large majority of the country. The period is between 20 and 30 days for roughly 80% of the total area; increases to 40 days and more are found toward the Gulf Coast and the NW Coast. Hence, one can anticipate that the periods of treatment for frost protection would be around 3 to 4 weeks per year at most locations. For uniformly distributed rainfall throughout the year, this would mean 6-8 % of the annual rainfall being potentially interfered with. The treatment periods would fall in Spring and Fall, with dates varying with latitude and altitude in well-known ways.

According to the second assumption, which appears to be not far from real possibilities in any regard, the duration of influence of frost-prevention treatments would be the entire period that the ground is not snow-covered. Since this is also the period of likely convective rainfalls, the potential seems to be appreciable that treatments could influence all meteorologically susceptible situations.

There are several missing links which prevent tying together the three factors discussed above in an effective manner. The precipitation statistics would have to be examined in detail in order to determine what fraction of the annual precipitation coincides with the period in which crop treatments might be applied, that is the extra frost-free period mentioned in the preceding paragraph. Similarly, the importance to crop development, not just the amount of that precipitation should be evaluated. Other economic impacts of that precipitation period should probably also be considered. Further, and probably more importantly, the fraction of annual and seasonal precipitation that is from convective clouds would have to be gauged somehow. Routine climatic data could not yield this information directly. Also, no generalizations are likely possible in this regard, since the relative contributions of stratiform and convective cloud systems are known to vary greatly by both location and season. Therefore, the data would have to be examined in detail. It should also be mentioned that changes in the ice nucleus concentrations entering a cloud will potentially influence the dynamics of the cloud as well as the development of precipitation. There are a number of connections between the initiation and growth of ice particles and the energetics of the cloud. The details of these links are best studied at the present by numerical models of clouds. Generalizations about the direction or magnitude of any changes in ice nucleus concentrations are not really warranted yet, just as there is considerable dialog about the projected outcome of seeding clouds.

The implicit assumption was made in the foregoing discussion that the result of reduced availability of active ice nuclei would be a reduction in the precipitation produced by the affected clouds. The opposite outcome, a possible increase in precipitation, can not be dismissed entirely, but it would take rather circuitous hypotheses to argue for such a result, since the role of the INA bacteria and of the biogenic soil nuclei is seen as initiators of precipitation at small supercoolings and in low concentrations.

The conclusions that appear warranted may be summarized as follows:

> The direct or indirect removal of INA bacteria from contiguous areas of a few hundred acres appears to constitute the threshold beyond which the development of precipitation might become affected. Beyond this threshold, the anticipated effect on convective rainfall would be roughly proportional to the fraction of land area over which nucleus concentrations are reduced. For the regions with large fractions of treated areas, a further effect, displaced hundred or more miles downwind, is likely to occur.

It should be reiterated that for specific sites the above statement can be refined to a considerable degree by a more detailed analysis of the available meteorological records.

D. Possible climatic impacts of ice nuclei.

The foregoing discussion focussed on the possibility of affecting precipitation by alterations of the ice nucleus populations on and around crops. Clearly this is the most immediate concern. Yet, there is another issue which should not be left untouched, namely the climatic impact of changed ice nucleus populations.

The physical basis for this connection is the importance of clouds in the thermal energy balance of the earth-atmosphere system as a whole. The transmission of radiation, both incoming and outgoing, is strongly influenced by clouds. In turn the microphysical evolution of clouds can influence their lifetimes and persistence in the atmosphere. Suffice it to say, that the concentrations of ice nuclei might influence the type and quantity of cloudiness over large areas and hence would impact on the radiation balance. Such changes may be imperceptible by most measures, because of the normally large variabilities in the atmosphere, yet an impact may develop over long periods of time.

The direction of change is not clearly predictable as there are processes linking ice nucleus concentrations to cloud persistence in both directions. Research work is very intensive on assessments of the climatic impacts of clouds. Conceivably the question of the influence of ice nuclei might be attacked by the coupling of the results of climate models with model predictions of the postulated changes in cloud evolution.

CHAPTER VII. TWO PLAUSIBLE, HYPOTHETICAL EXAMPLES OF THE POTENTIAL IMPACT OF MODIFICATIONS OF INA BACTERIA ON ATMOSPHERIC PROCESSES.

The number of imponderables and unknowns discussed in chapters III, IV, V and VI reveal more what we do not know than what we do. We have identified a number of things that we think might happen, but have not narrowed the possibilities to simple conclusions. Our analyses have been in terms of the economic variables, scientific unknowns, and technological questions posed. The diversity of the physical and biological environments and our ignorance of the extent to which they may interact on a continental scale make development of a plausible scenario on that scale beyond the scope of this report, if not current feasibility. Yet, on more modest scales it is feasible to make educated guesses as to the sort of thing that may happen.

In this chapter we will develop plausible scenarios for two relatively small regions with a tentative assessment of the impact on precipitation that may accrue were the two scenarios to come to pass. It is our hope that these examples may be sufficiently explicit to better illustrate the potential impact of use of the technologies described in chapter V. They also serve to focus attention on how weak the assumptions are that had to be made in order to arrive at the conclusions that we have drawn; the extent that these assumptions can be tested is summarized in chapter X.

The analyses presented in these scenarios should be regarded as preliminary. A thorough analysis of existing data could undoubtedly refine the results, and provide a more comprehensive picture of each of These two possibilities. However, a detailed analysis of this type is beyond the scope of this report.

A. Protection against fall frost in a northern part of the corn belt.

Assumptions:

1. Crops, growing conditions and economic conditions.

We will consider an area about 75 km in radius on the Illinois - Wisconsin border. About 50% of the land area is planted to frost sensitive crops, principally corn, but including substantial acreages of soybeans as well. The remainder of the land area is covered with plants that support INA bacteria. It has been a very difficult growing season throughout the midwest. Relative to the rest of the corn belt, the crops in the area under consideration are in fairly good condition, but are later than usual, due to delays in spring planting. Maturity of the corn crop is about ten days later than normal; soybeans are nearly two weeks behind normal. USDA crop forecasts are for a much smaller crop of all feed grains than has been the case for several years. The prices of corn and soybeans are very high.

Taken together, the above assumptions might justify the conjecture that if there were a technology available that could decrease the temperature at which frost injury would occur by as little as 0.5 C, at a cost of $15/acre or less, it would be applied to about 70% of the corn and soybeans, or about 35% of the land area.

2. Available technologies.

We will assume that each of the four possible technologies has been developed to the extent that it can be utilized to decrease the temperature at which frost injury to corn and soybeans occurs by about 0.5 C or more.

a. Ice nucleation inhibitors.

Materials will have been identified that are effective ice nucleation inhibitors, are safe in the environment, are not phytotoxic, are not toxic to humans and can be safely manufactured and applied to crops. These ice nucleation inhibitors will be applied to all of the crops in the area under consideration to be treated (e.g. 35% of the land area).

b. Bactericides.

Materials will have been identified that specifically kill or inhibit the growth of INA bacteria, other epiphytic bacteria are not affected. These materials will have passed all of the necessary requirements for registration as a phytobactericide for use on both corn and soybeans. One or more of the bactericides with these properties will be applied to 70% of the corn and soybeans in the area under consideration. Multiple applications may be necessary at seven to ten day intervals, beginning the first or second week in September to achieve sufficient decreases in bacterial population sizes and allow time for decay of existing bacterial ice nuclei. Application will continue until the crops are eventually killed by frost or until they are sufficiently mature that yields will not be affected by frost. This will be assumed to be about 20 October.

c. GEMs for preemptive exclusion.

GEMs will have been developed that are effective in excluding INA bacteria from becoming established on both corn and soybeans. We expect that to do this a different bacterium will be used that is specific for each of these crops. Each of these two bacteria will be registered for use as a biological pesticide on its respective crop. Application of the bacteria will have been made early in the growing season, before natural populations of INA bacteria became established on the crops. The initial application may have been made to the seeds; additional foliar applications will probably have been required to maintain the competitor in a sufficiently dominant population. For effective protection against frost, normal populations of INA bacteria have not been allowed to build up all season long.

The competitors are assumed to have spread successfully to nearby vegetation. Thus, the numbers of INA bacteria have been affected on vegetation equivalent to about twice the area treated. Numbers of bacterial ice nuclei may have been decreased by 100-fold on an area corresponding to 70% of the area under consideration.

d. Plant cultivars that select against growth of INA bacteria.

Commercially acceptable cultivars of soybeans, and hybrids of corn have been developed that do not allow populations of INA bacteria to build up on their leaves. These plants support about 1000-fold lower INA bacterial population sizes than plants of the same species that lack this property. Plants of this type have been planted to 70% of the corn and soybean fields in the area in question. Numbers of INA bacteria on these fields have been three orders of magnitude lower than normal all season long.

In summary, the following assumptions are made for this scenario:

Treatment	Area	INA reduction	Period
a. Inhibitors	35%	100-fold	Sept-Oct
b. Bactericides	35%	100-fold	Sept-Oct
c. GEMs	70%	100-fold	May-Oct
d. Host genotype	35%	1000-fold	May-Oct

An additional assumption one could make is that the treatments have been applied by now for over several years so that the soil in the treated areas have much lower bacteria-derived nuclei for treatments c and d; for the other treatments no such long-term effect is expected to develop.

3. Analysis

The precipitation records for the site can be characterized by the average monthly precipitation over 20 years, also averaged for the four State Climatic Divisions bracketing the Illinois-Wisconsin border (USDC, 1968):

May - 3.61"   June - 4.56"   July - 3.52"
Aug - 3.52"   Sept - 3.44"   Oct - 2.37"

It is seen that there is little change in rainfall over the summer months; only by October is there a notable reduction. The total for the six-month period is 21.02" and for the last two months 5.81". The variability of the monthly rain amounts can be characterized by saying that 3 or 4 years out of 10 will differ from the mean by less than 1".

Meteorological classifications of rain events in Illinois have been developed by Hiser (1956) and by Vogel and Huff (1977), and are summarized by Changnon and Huff (1980). The two available studies incorporate different classification schemes and are therefore difficult to reconcile. During a ten year period, Hiser found that frontal activity accounted for 74% of the July-August rainfall at four stations. Cold fronts were associated with 39% of the rainfall. Vogel and Huff analyzed rain events for a five-year period in the St. Louis, MO. area and ascribed 76% of the June-August rainfall to mesoscale "squall line" and "squall zone" systems, while direct frontal activity was diagnosed for only 20% of the rainfall. Both studies agree on the overwhelming importance of convective cloud formations for the summer months. While it seems likely that this finding can not be simply extended into the September to October period, for simplicity this possible difference will not be dealt with here.

The dominant precipitation process in summer cumuli of the mid-Western U.S. has been deduced to be the warm-rain mechanism. The broadest basis for this conclusion was the examination of the altitudes of radar echo initiation in Ohio, Missouri and in the southern and central parts of Illinois (Battan, 1953; Braham, 1964; Johnson and Dungey, 1978). Aircraft sampling in Missouri tended to support the conclusion (Koenig, 1963) but in post-frontal cumuli of Illinois evidence was found for the possible importance of the ice-phase process (Sax, Eden and Ackerman, 1978). Also, just to the north of Lake Superior the development of precipitation was found to depend on ice initiation, and could be promoted by seeding with ice nuclei (Isaac et al., 1982).

Even with the information summarized above there is a great deal of latitude in how to address the question of possible impact due to the removal of INA bacteria from crops. The predominance of convection certainly argues that surface sources of ice nuclei could play a role in rain initiation, but the fraction of clouds for which this might be important is difficult to state. A minimum estimate, based on the Sax et al. (loc. cit.) finding, is to consider the post-frontal clouds only; this would reduce the possible impact to clouds that normally produce only about 1.5% of the summer rainfall according to the data of Hiser (1956). However, the possibility that ice nuclei are at least of some influence on precipitation formation in a large fraction of cases is supported by the fact that the average maximum echo heights are at temperatures of -10 C or colder all through the summer (Changnon and Morgan, 1976), and by the proximity of the site in question to the area studied by Isaac et al. (loc. cit.). Thus, reduced to having to make guesses, we will assume that 30% of the rainfall is from clouds which would have altered precipitation efficiencies if the ice nucleus flux to the cloud were reduced.

The size of the area considered in this scenario is large enough to assume that clouds are affected in their entirety with some clouds spending their total lifetime over the modified area and others only a part, depending on their points of origin. The treated areas are assumed to be in patches which are small compared to the updraft areas of the clouds. Hence, each cloud is receiving a mix of air from over treated and non-treated patches. The resulting reductions in nucleus concentrations are: 35% for treatments a, b and d, and 70% for treatment c. With a 5 C per decade slope for the ice nucleus spectrum, these changes in concentration correspond to shifts in the temperature at which the effects of the nuclei are manifested in the clouds by 1 C and 2.5 C respectively. In lieu of detailed numerical models of the applicable cloud processes, the changed nucleus concentrations are assumed to result in 10% and 20% reductions in precipitation efficiency for the two cases.

The results of the assumed proportions of susceptible clouds and the (also assumed) changes in precipitation efficiency translate into reductions in the amount of precipitation by 3% over September and October for treatments a and b, by 3% over May to October for treatment d, and by 6% over May to October for treatment c. These changes are small compared to inter-annual variability and so would be undetectable and unnoticed even if the change persisted over many years. Average summer rainfall in the area is sufficient for production of ample crops. There is no economic impact expected from the amount of rainfall reduction. The frequency of rain, however is sufficiently variable that crops do experience periods of insufficient moisture in many years. The area of reduced rainfall is just the area of treated crops, as a first approximation; a 10-km displacement toward the East is a somewhat better approximation.

One should not loose sight of the possibility that the aforesaid results assume linear averaging of the impacts of INA bacteria on the clouds. The analysis is very sensitive to the affect of patch size relative to cloud area. With larger patches of treated crops in this situation, small to medium convective cells will experience full 100 or 1000-fold reductions in nucleus fluxes for all or part of their lifetimes. This may lead to a reduction in the proportions of light to medium rains, by having some of these clouds stay below the thresholds of precipitation formation, and an overall reduction in rainfall which is larger than that estimated with the averaging. If these reductions occur during periods when moisture is limiting crop development, then the overall effect could cause an amplification of the effects of droughty periods on agriculture.

If treatments c and d have been applied over several years than the potential exists for rainfall over the entire snow-free period to be influenced. Considering reduced expected impact for non-convective clouds and the shift to such clouds outside the Summer period, only a small additional effect is postulated for April and November.

The area in question has a point frequency of hailfall of about 3 per year. Hail is therefore a definite consideration for the area, but the general statement of chapter VI can not be refined - it is possible that the severity or frequency of hail might change and the change may be in either direction.

In summary, considering an averaged effect, the removal or reduction of INA bacterial populations, even with a long-term cumulative effect on the soil nuclei, is not expected to produce "noticeable" reductions in rainfall in the situation here envisaged. On the other hand, reductions in precipitation from smaller systems may have a noticeable effect on precipitation during periods of insufficient moisture. Potentially important impacts on hail frequency or hail size, and on the frequency of smaller showers should not be ruled out.

B. Protection against January frost in southwestern Florida.

Assumptions:

1. Crops, growing conditions and economic conditions.

The area in consideration extends from Fort Meyers about 40 miles south to the Everglades, and from the gulf about 35 miles inland. Nearly 30,000 acres of high-value crops are planted in this area. About half of the acreage is tomatoes, the remainder includes cucumbers, squash, peppers, and melons. Small areas of other frost sensitive crops such as ornamentals and seed corn may also be planted in the area.

The winter vegetable growing area of the west coast of Florida is frequently subjected to economically important frosts in which the temperatures are only slightly below freezing. As little as 0.5 to 1.0 C of protection would frequently prevent substantial damage to these crops. Growers in this area consider frost a major limitation to production.

All crops in the ground during January are of sufficient value that any available technology to decrease frost damage would be implemented.

2. Available technologies:

Frost prevention technology assumptions are the same as for case A (above), except that they have been adapted for use on all of the crops in the southwest Florida area.

3. Analysis

The precipitation records for the month of January show roughly 1.5" average rain. There is a large amount of annual variability: 2 out of 10 years may have rainfalls 1/3 as small or twice as large as the average.

While the summer cloud systems of southern Florida are among the best-studied ones in the U.S., thanks to the Florida Area Cumulus Experiment, very little information is readily found on the winter situation. The climatological data reveal that the lower precipitation amounts of the winter months are associated with fewer days of rain exceeding 0.1", but the number of days is 1/3 while the rainfall is 1/5 of the summer months. The fraction of raindays with thunderstorms is 1/5 in January, whereas it is 1 or greater in the summer. These comparisons demonstrate that the cloud populations, if not the types, are different for January than for the well-studied summer months.

In view of the aforesaid, the estimation of the impacts of treatments for the removal of INA bacteria is a nearly total guesswork for this scenario. On the other hand, the small fraction of the site that is treated (3.3%) makes it unimportant what the cloud characteristics are. Treatment of 3.3% of the land area, with an attendant elimination of the INA^+ bacteria, but with the other 96.7% of the land area still bearing the native populations of INA^+ bacteria, and assuming that normally the two populations would be of equal strength and equally likely to be released into the atmosphere, can be taken to result in a 3.3% reduction in the available ice nuclei when averaged over the whole area. Since the sizes of the plots of frost-sensitive plants are on the order of 1 to

10 km, the areas of reduced nucleus sources are comparable to the updraft areas of small to medium-sized convective clouds. Therefore, these clouds will be affected most seriously, and larger ones will be affected less. To estimate the resulting changes in precipitation probabilities the rough estimate of 1/3 each seems reasonable for the categories (i) clouds of comparable size to the plots and hence significantly affected by the absence of nuclei, (ii) clouds larger than the plots but convective and hence partially influenced, and (iii) clouds that are unaffected. With this situation, the overall reduction in precipitation over the area, or near it may amount to a fraction, or perhaps 1 percent, when averaged over the area and season in question. There may be days with reductions of twice or three times the average due to the preponderance of cloud sizes most sensitive to the reductions of nucleus concentrations and the total failure for precipitation to develop from a few percent of the clouds. Days with very heavy rainfall are likely to be influenced to a minimal extent.

The impacts of the small reductions in rainfall are not likely to be appreciable for years with near normal precipitation. In the SW Florida case, because of the highly variable annual precipitation, there is a concern about even small reductions in rainfall if they come on top of already unusually low rainfall in a given year. There is a reasonable chance that in those years the available clouds are more sensitive to the concentrations and activity of ice nuclei than in more closely normal years. This, of course, would further the danger of a serious impact.

In summary, the Florida scenario shows that the contemplated treatments would be inconsequential for years with near normal precipitation, but that in as many as one in five years, with only 1/3 normal precipitation, there could be serious concerns about further reductions in rainfall. Also, a shift in the relative proportions of light to heavy rains might occur, with yet unassessed consequences.

C. Summary.

Overall, consideration of the likely effects on precipitation of frost prevention technologies in these two very different climates have lead us to the following conclusions. First, although there may be an effect on the average amount of precipitation, the amount of this reduction will be so small relative to the normal variability in precipitation that it will be difficult to measure. Second, the effect on the frequency of precipitation during dry periods particularly, from small systems, may be larger than the effect on average precipitation. Third, changes in the proportion of precipitation associated with heavy showers or light showers, and of hail are likely to occur. Finally, although the largest sources of uncertainty in our analysis reflect large gaps in what is know about this important area, a more thorough analysis of the effect of decreasing numbers of ice nuclei on precipitation production in both areas is possible using current data.

CHAPTER VIII. OTHER POTENTIAL ECOLOGICAL IMPACTS OF LARGE SCALE AGRICULTURAL APPLICATIONS OF ICE-MINUS BACTERIA

Some respiratory diseases, which are thought to be immune in nature, have been associated with crop dusts (farmer's lung) or, specifically, cotton dust (byssinosis). In both cases, there is some evidence that *E. herbicola*, or other plant associated bacteria may be involved (Millner, 1984). If selected bacteria, genetically modified or not, are applied to large acreages of crop land in large numbers to outcompete INA bacteria, the total numbers of bacteria on these crops, or numbers of the species applied are likely to be increased. Since the current thinking is that the best competitors are probably non-nucleating members of the same species that produce ice nuclei, numbers of these species are likely to be elevated on the crops to which they are applied. If the bacteria that are applied are antigenically comparable to the wild type bacteria that they are used to overwhelm, then numbers of bacteria capable of triggering these diseases may be increased on the treated crops.

CHAPTER IX. ARE THERE LIKELY TO BE INDIRECT ECONOMIC OR SOCIAL IMPACTS OF THIS KIND OF TECHNOLOGY?

A. Will the technology increase overproduction in agriculture?

The extent to which cropland in the U.S. will be expanded by avoiding light frosts is probably not very large. Thus, in this country at least, the total acreage under cultivation will probably not be much affected. Most land that is suitable for production of crops, but is subject to summer frosts, is currently being used for production of crops that are unlikely to be damaged by frost, or being used at least for pasture.

If, however, growers in several areas of the country expect that they can extend their growing season by as little as two weeks each spring and another two weeks each fall, or completely remove the threat of midsummer (in the north) or midwinter (in the south) frosts, the kinds of crops that they raise, or the time that they plant them may be substantially influenced. Merely avoiding frost is not enough in itself to affect a drastic change in the location in which crops are grown. Many seeds will not germinate in cold soils, whether or not the seedlings would be killed by frost. Thus, these crops will not be planted until the warm part of the growing season has arrived and the soil is warm enough. Preventing frost injury will not extend their range. Many crop plants are severely damaged by chilling injury at temperatures substantially above freezing. Thus, much more must be considered by a grower who wishes to plant a crop in an area where or when it would be killed by frost without removing INA bacteria. However, there are some cases where, in the minds of some growers, the economic advantage of trying it is likely to outweigh risks. The effects on total production are likely to be very small. In a few locations, crops may become profitable that are currently too risky, but these are likely to be high value, low acreage crops, like winter vegetables. Large new acreage of major crops should not be expected.

B. Where new uses of land are encouraged, might there be new, unexpected risks to the agricultural community?

If we are to decrease the temperature at which frost injury becomes likely by 1 C, some crops are likely to be grown on sites that are not now considered suitable for their culture. Although the total acreage so displaced will probably be very small nationally, it is likely to be clustered in a few areas. Thus, there could be substantial agricultural communities that rely on this type of technology. We can foresee at least two risks that these communities would face that today's growers do not. First, microorganisms can (and do) develop resistance to treatments to prevent their damage to crops. Resistance to pesticides is quite common, and can develop very rapidly in a population of bacteria. New genotypes of plant pathogens frequently occur to overcome genes for resistance incorporated into crops by plant breeders. We expect that competing bacteria may present a very strong selective environment for genotypes of INA bacteria that can overcome the biocontrol agent. Thus, it is quite likely that any given technology for protection of crops against frost will eventually fail in the presence of new types of INA bacteria. Such a failure could be disastrous to an agriculture that was completely dependent on this technology.

The other risk is to frost damage when the 1 C protection is fully functional. Many growers, all over the country, occasionally incur some crop losses to frost injury. It is simply one of the many risks of farming. If growers are willing to accept occasional frost injury, say one frost in ten years, and if reliable technology is available for them to move to a new site where the probability of frost exactly 1 C lower is only once in ten years, then where is the increased risk? The severity of damage from a -2 C event is likely to be limited by the amount of freezing that actually occurs in plant tissues. After ice nucleation occurs, much heat must be removed from the crop canopy in order to freeze enough water to cause a given level of damage. This is due to the substantial latent heat of fusion of ice. Rates of heat transfer are roughly proportional to temperature gradients. As the ice to water phase transition occurs in dew on leaves, or even in some tissues, the tissue temperature will be 0 C, the melting point of water. At -2 C, the temperature gradient is only about 2/3 as large as it is at -3 C. Thus, the rate of heat removal and hence, ice spread in the canopy is much slower in the frost event that occurs at the warmer temperature. Thus, the overall extent of injury from a frost event that occurs at -3 C will probably be much greater than from an event that occurs at -2 C, even if the amount of ice nucleation that occurs in the canopy is the same. Under today's practice, the one frost in ten years may only cause yield or quality loss, if it occurs at the warmer temperature. If "frost protection" technology is used, frost at the lower temperature may cause a more nearly total loss.

CHAPTER X. RECOMMENDATIONS.

This chapter summarizes, in the form of recommendations, those things that we believe should be done to improve our knowledge of the true hazards involved in attempting to control frost injury to crops by modifying the population biology of the epiflora on those crops. Throughout this report, we have had to make a number of assumptions, some on rather weak grounds, in order to proceed with our analysis of what may happen. Many of these assumptions can be restated as testable hypotheses, and approached empirically. For some, analysis of existing data would be adequate; for others experiments must be performed to gather new data. The importance of performing the necessary research is underscored by the current availability of one phytobactericide for frost protection. This material is not very effective, and can only be used on a limited number of crops due to its phytotoxicity. We have no assurance, however, that the next entry into the marketplace will not be more effective and more generally utilizable. Thus, there is currently a little time for necessary consideration of the issues presented in this report. But only a little. If we, as a community, fail to fund and perform the necessary research to properly investigate the potential hazard of any of these technologies for protection against frost injury, then we, as an economy will. Since the latter experiment may contain fewer safeguards, and exercise less control, our preference would be for the scientific approach.

A. Short-term actions.

There are a number of ways that the assessments assembled in this report could be improved with relatively modest expenditures of effort. These are in areas where data are already available to answer some questions, but where processing and interpretation of the data required more time than was available for the preparation of this report.

1. Estimates of the fraction of precipitation produced by different cloud types. It was argued in chapter VI.A. that the importance of ice nuclei for precipitation production in general is likely to vary with cloud type, and in chapter VI.C. the impact of reduced concentration of INA bacteria on plants was shown to be most immediate on convective precipitation. Hence, available precipitation records should be examined, in conjunction with other meteorological records, to estimate the proportion of precipitation that is received at different geographical locations and at different times of the year from convective clouds. Since different size clouds will experience different impacts, in relation to the patch sizes of treated areas, data should be examined with respect to the frequency distributions of cloud sizes (perhaps as radar echo distributions). "Patch size", or size of contiguous area planted to specific crops can be obtained by analysis of aerial photos, available through county ASCS offices for many areas of the country.

2. The fraction of precipitation that falls during periods when treatments of crops for frost prevention might be contemplated need to be evaluated. Whatever fractional reduction in precipitation is estimated to be likely, it is only the precipitation that falls during the periods

of successful treatment that is going to be affected. The period of influence on rainfall will extend to all snow-free periods only under two conditions. First, if the relationship between organic ice nuclei in soil and the INA bacteria on plants is confirmed, and if treatments over several years are contemplated, then ice nuclei available during periods when neither snow nor a crop canopy cover the soil may be affected. Or if the treatment affects numbers of INA bacteria on plants other than those that are actually treated, the effect may be expected to extend, at least to some degree, to periods when the treated crop is no longer standing. Data for this analysis are readily available. Since some of the potential treatments will have season-long effects, while others may be used only at times that frost is actually expected, this analysis will be sensitive to the nature of the treatment to be used.

3. The climatological data with respect to the economic gains due to protection against frost injury that may accrue from the reductions of INA^+ bacterial populations have to be examined in greater detail. Data used in this report were taken from the published records for randomly selected stations and were used without taking into account the altitudes of the sites and other special characteristics. These corrections could have fairly major effects on the results. Also, a temperature change (for frost damage occurrence) of 2 C was considered; smaller changes are more likely to be produced in reality, so the changes in frost probability have to be reevaluated for those temperatures.

4. The impacts of changes in precipitation amounts have not been evaluated in this report. The economic value of precipitation is highly

variable by location, timing, etc., so the evaluation of this factor in the estimation of cost/benefit ratios for the treatment of crops for frost prevention requires attention.

B. Research goals.

The many caveats and qualifications that had to be accepted in the statements of the preceding chapters point out the undeveloped status and cross-disciplinary nature of the problems being addressed. It is clearly evident that there are a number of research requirements which should be attacked in order to remove serious roadblocks to progress. We list here some of these avenues, both to have in record and to put the present form of the assessment into better perspective.

1. The most crucial untested link in the relationship between crop treatment for frost protection and precipitation is the role that INA bacteria play in the initiation of precipitation. Not only is the quantitative relationship unknown, but there really is no direct evidence yet available for either the presence or the active role of these bacteria in the formation of precipitation. To fill this gap, there seems to be no alternative but to undertake an observational program designed specifically for the examination of this question. There are considerable difficulties to be overcome in such an experiment, which is the reason for not yet having had the experiment performed, but there are reasons for optimism that the problems can be solved with appropriate effort. The main lines of approach to the problem seem to be observational programs and randomized experiments. It would seem possible to have answers within about 3 to 5 years. The complexity of

the problem requires the participation of several research groups from very different scientific disciplines. Funding from a number of agencies is likely to be necessary.

2. The relationship between organic ice nuclei in soils and the INA bacteria on plants is yet unknown. This is a very important problem and needs experimentation both in the laboratory and at a variety of field sites.

3. If some of the organic ice nuclei in soils are due to or derived from epiphytic INA bacteria, then the role of these active products that are found in soils is potentially even more important than the cells themselves, at least in some locations and seasons. This is a tougher problem, because of the unknown nature of these nuclei, and hence the difficulty of identifying them. Progress may be possible in this area by the examination of nuclei fluxes from the ground into the air in relation to the availability of nuclei in the soils. This study, and in part the preceding one also, depends on the availability of a device capable of detecting and counting airborne nuclei active at temperatures near -5 C. No such device exists at the moment, and its development is likely to take several years.

4. The application of numerical cloud models to the estimation of effects due to changed concentrations of ice nuclei should be encouraged. A number of models exist which could be used for such studies; in fact it is advisable to test and compare the results from several models.

5. If bacterial ice nuclei are shown to participate in cloud processes, then the relative importance of the various plants that constitute the major fraction of the ground cover over the country in producing these bacteria needs to be known. A serious effort should be made to determine the relative population sizes of INA bacteria on the various plants that are major constituents of ground cover in each region of the country.

6. The response of growth of INA bacteria to weather and plant phenology should be examined in several different types of climate in the country.

7. The likelihood of aerial dispersal of INA bacteria from different types of plants under different weather conditions is necessary before the relative importance of bacteria resident on different plants to atmospheric processes can be evaluated.

8. Relative selection for bacterial genotype by plant genotype must be evaluated quantitatively. That plants tend to select for bacterial genotype is only known as a qualitative phenomenon at the present. The relative importances of growth on a particular plant and immigration to that plant can only be properly evaluated if this phenomenon can be understood quantitatively. This needs to be accomplished for conspecific populations to evaluate the likely impact due to spread of GEMs, and for naturally competing species to evaluate the impact on INA bacterial population sizes on non-treated plants of the use of bactericides and plant genetics for frost protection. Only after adequate quantitative evaluation of this kind of selection will it be possible to assess the

real danger posed by spread of GEMs or of the other non-nucleating bacteria that may be selected from by plants that select against INA bacteria.

TABLE 1. Plant taxa reported or assumed to harbor INA *P. syringae*, *P. fluorescens*, *P. viridiflava*, *E. herbicola*, and *X. campestris*.

Plant species	Bacterial species*	References
Herbaceous plants		
Alfalfa (*Medicago sativa* L.)	Ps, Pv, Eh	Lindow et al., 1978; Lukezic et al., 1983
Amaranthus sp. L.	Ps, Eh	Lindow et al., 1978
Asparagus (*Asparagus officinalis* L.)	Ps	Lindow et al., 1978
Banana (*Musa* L. sp.)	Ps, Eh	Lindow et al., 1978
Barley (*Hordeum vulgare* L.)	Ps, Xct	Lindow et al., 1978
Bean (*Phaseolus vulgaris* L.)	Ps, Pv, Eh	Lindow et al., 1978; Hirano et al., unpub
Beet (*Beta vulgaris* L.)	Ps, Eh	Lindow et al., 1978
Daffodil (*Narcissus pseudonarcissus* L.)	Ps	Lindow et al., 1978
Cauliflower (*Brassica olearacea* L.)	Ps, Pv	Lindow et al., 1978; Wilkie et al., 1973
Chrysanthemum (*Chrysanthemum* x *morifolium* Ramat)	Ps, Eh	Lindow et al., 1978
Clover, red (*Trifolium pratense* L.)	Ps, Pv, Eh	Lindow et al., 1978; Lukezic et al., 1983
Cucumber (*Cucumis sativus* L.)	Ps, Eh	Lindow et al., 1978
Dandelion (*Taraxacum officinale* Wingers)	Ps, Eh	Lindow et al., 1978
Geranium (*Pelargonium* x *hortorum* L. H. Bailey)	Ps, Eh	Lindow et al., 1978
Gooseberry, Chinese (*Actinidia chinensis* Planch.)	Pv	Wilkie et al., 1973
Grass, Kentucky bluegrass (*Poa pratensis* L.)	Ps	Lindow et al., 1978
Grass, quackgrass (*Agropyron repens* (L.) Beaurois)	Ps, Eh	Lindow et al., 1978
Lettuce (*Lactuca sativa* L.)	Ps, Eh	Lindow et al., 1978
Lupin (*Lupinus angustifolius* L.)	Pv	Wilkie et al., 1973
Maize (*Zea mays* L.)	Ps, Eh	Lindow et al., 1978
Marigold (*Tagetes patula* L.)	Ps, Eh	Lindow et al., 1978
Muskmelon (*Cucumis melo* L.)	Ps, Eh	Lindow et al., 1978
Mustard (*Brassica hirta* Moench.)	Ps	Lindow et al., 1978
Oat (*Avena sativa* L.)	Ps	Hirano et al., 1981
Onion (*Allium cepa* L.)	Ps	Lindow et al., 1978
Parsnip (*Pastinaca sativa* L.)	Pv	Hunter & Cigna, 1981
Passionfruit (*Passiflora edulis* Sims.)	Pv	Wilkie et al., 1973
Pea (*Pisum sativum* L.)	Ps	Lindow et al., 1978
	Pv	Wilkie et al., 1973

Plant species	Bacterial species*	References
Pepper (*Capsium frutescens* L.)	Ps, Eh	Lindow et al., 1978
Phyllostachys sp. Sieb. & Zucc.	Ps, Eh	Lindow et al., 1978
Poinsettia (*Euphorbia pulcherrima* Willd)	Pv	Suslow & McCain, 1981
Poppy (*Papaver somniferum* L.)	Pv	Wilkie et al., 1973
Potato (*Solanum tuberosum* L.)	Ps, Eh	Lindow et al., 1978
Primrose (*Oenothera biennis* L.)	Ps, Eh	Lindow et al., 1978
Pumpkin (*Cucurbita maxima* Duchesne)	Ps, Pv, Eh	Lindow et al., 1978; Wilkie et al., 1973
Radish (*Raphanus sativus* L.)	Ps, Eh	Lindow et al., 1978
Ragweed, great (*Ambrosia trifida* L.)	Ps	Lindow et al., 1978
Rye, winter (*Secale cereale* L.)	Ps	Lindemann et al., 1984
Soybean (*Glycine max* (L.) Merrill)	Ps, Eh	Lindow et al., 1978
Strawberry (*Fragaria chiloensis* (L.) Duchesne)	Ps, Pf, Eh	Lindow et al., 1978; Lindemann
Thistle, sow (*Robinia pseudoacacia*)	Ps	Lindemann et al., 1984
Trefoil, birdsfoot (*Lotus corniculatus*)	Pv	Lukezic et al., 1983
Tomato (*Lycopersicon esculentum* Mill.)	Ps, Pv, Eh	Lindow et al., 1978; Jones et al., 1984
Turnip (*Brassica rapa* L.)	Ps, Eh	Lindow et al., 1978
Vetch, hairy (*Vicia villosa* L.)	Ps	Lindemann et al., 1984
Watermelon (*Citrullus lanatus* (Thunb.)Matsum. & Nakai	Ps, Eh	Lindow et al., 1978
Wheat (*Triticum aestivum* L.)	Ps, Eh	Lindow et al., 1978
Zinnia (*Zinnia elegans* Jaeg.)	Ps, Eh	Lindow et al., 1978
Woody plants		
Alder (*Alnus tenuifolia*)	Ps	Maki et al., 1974
Almond (*Prunus dulcis* (Mill.) D.A. Webb	Ps	Lindow et al., 1978
Apple (*Malus sylvestris* Mill.)	Ps, Eh	Lindow et al., 1978
Apricot (*Prunus americana* Marsh.)	Ps	Lindow et al., 1978
Ash, American mountain (*Sorbus americana* Marsh.)	Ps	Lindow et al., 1978
Aspen, Quaking (*Populus tremuloides* Michx.)	Ps, Eh	Lindow et al., 1978
Umbrella tree (*Brassaia actinophylla* Endl.)	Ps, Eh	Lindow et al., 1978
Boxelder (*Acer negundo* L.)	Ps	Lindow et al., 1978
Cedar, red (*Juniperus virginiana* L.)	Ps, Eh	Lindow et al., 1978
Cherry, black (*Prunus serotina* J. F. Ehrh.)	Ps, Eh	Lindow et al., 1978
Cherry, choke (*Prunus virginiana* L.)	Ps	Lindow et al., 1978
Cherry, sour (*Prunus cerasus* L.)	Ps, Eh	Lindow et al., 1978
Cherry, sweet (*Prunus avium* (L.)L	Ps, Eh	Lindow et al., 1978

Plant species	Bacterial species[*]	References
Citrus sp.	Ps, Pv, Eh	Lindow et al., 1978; Stall, per comm.
Crape-myrtle (*Lagerstroemia indica* L.)	Ps, Eh	Lindow et al., 1978
Currant (*Ribes* L. sp.)	Ps, Eh	Lindow et al., 1978
Elderberry (*Sambucus canadensis* L.)	Ps, Eh	Lindow et al., 1978
Fir (*Pseudotsuga menziesii* (Mirb.) Franco)	Ps	Lindow et al., 1978
Fir, balsam (*Abies balsamea* (L.) Mill.)	Ps, Eh	Lindow et al., 1978
Grape (*Vitis vinifera* L.)	Ps, Pv, Eh,	Lindow et al., 1978; Wilkie et al., 1973
Grape (*Vitis riparia* Michx.)	Ps, Eh	Lindow et al., 1978
Hickory (*Carya ovata* (Mill.) C. Koch	Ps, Eh	Lindow et al., 1978
Lilac (*Syringa vulgaris* L.)	Ps	Lindow et al., 1978
Locust, black (*Sonchus arvensis* L.)	Ps	Lindemann et al., 1984
Magnolia (*Magnolia virginiana* L.)	Ps, Eh	Lindow et al., 1978
Oak, burr (*Quercus macrocarpa* Michx.)	Ps, Eh	Lindow et al., 1978
Oak, red (*Quercus ruba* L.)	Ps, Eh	Lindow et al., 1978
Peach (*Prunus persica* (L.) Batsch.	Ps	Lindow et al., 1978
Pear (*Pyrus communis* L.)	Ps	Lindow et al., 1978
Plum (*Prunus domestica* L.)	Ps, Eh	Lindow et al., 1978
Poplar (*Populus angustifolia* James)	Ps	Lindow et al., 1978
Raspberry (*Rubus idaeus* L.)	Ps	Lindow et al., 1978
Shad bush (*Amelanchier arborea* (Michx.f.) Fern.)	Ps	Lindow et al., 1978
Walnut, black (*Juglans nigra* L.)	Ps, Eh	Lindow et al., 1978
Walnut, English (*Juglans regia* L.)	Ps	Lindow et al., 1978

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[*] Ps = *Pseudomonas syringae*, Pf = *Pseudomonas fluorescens*, Pv = *Pseudomonas viridiflava*, Eh = *Erwinia herbicola*, Xct = *Xanthomonas campestris* pathovar *transluscens*. Literature cited do no reflect original description of the particular bacterial species on a given plant species. If the researchers conducted tests to determine whether the bacterial isolates were ice nucleation active, the publication is listed above, although the presence of the bacterial species on that particular plant species had been described previously in the literature. Ice nucleation activity was not determined in all of the studies cited above.

TABLE 2. 1982 UNITED STATES LAND UTILIZATION

State	Crop Land	Coniferous Forest	Total Land Area	*Total Area Coniferous Forest
ALA	5605	16.1	32491	32474.9
ARIZ	1553	819.8	72645	71825.2
ARK	10641	17	33330	33313
CALIF	11559	6418	100031	93613
COLO	10999	2544.1	66301	63756.9
CONN	234	19	3118	3099
DEL	542	0	1236	1236
FLA	4621	255.1	34658	34402.9
GA	7205	58.3	37156	37097.7
HAW	347	0	4112	4112
IDAHO	6672	6717.9	52744	46026.1
ILL	25773	5.3	35613	35607.7
IND	13873	1.5	22996	22994.5
IOWA	28683	0.2	35818	35817.8
KANS	33030	0	52338	52338
KY	9620	12.8	25388	25375.2
LA	6469	58.3	28493	28434.7
MAINE	627	700	19837	19137
MD	1872	26.4	6296	6269.6
MASS	282	40.3	5007	4966.7
MICH	9006	301	36451	36150
MINN	23515	1361.1	50911	49549.9
MISS	8259	6.4	30229	30222.6
MO	20158	48.7	44125	44076.3
MONT	17098	6742.5	93048	86305.5
NEBR	24538	37.4	49052	49014.6
NEV	900	1457	70332	68875
N H	165	764	5756	4992
N J	691	13.2	4779	4765.8
N MEX	2362	1313.8	77654	76340.2
N Y	5889	842.9	30321	29478.1
N C	6495	61.9	31260	31198.1
N DAK	30900	0	44352	44352
OHIO	12315	3.6	26243	26239.4
OKLA	15453	12.8	43939	43926.2
OREG	5386	2467	61558	59091
PA	5710	9.1	28728	28718.9
R I	36	0	675	675
S C	3544	5.5	19330	19324.5
S DAK	20538	104.9	48609	48504.1
TENN	8164	89.4	26339	26249.6
TEX	39775	44.5	167691	167646.5
UTAH	2003	1174.9	52527	51352.1
VT	819	42.7	5935	5892.3
VA	4982	30.6	25411	25380.4
WASH	8394	5008	42567	37559
W VA	1470	37.8	15436	15398.2
WIS	12475	243	34833	34590
WYO	2841	4688.5	62073	57384.5
U S	474178		1899812	1855149.7

Unit = 1000 acres; Agriculture Statistics

*Potential area for plants bearing Ice nucleation active bacteria.

TABLE 3. 1984 STATE AREA PLANTED TO FROST SENSITIVE CROPS.

	Corn	Sorghum	Durum Wheat	Spring Wheat	Soybean	Sun-Flower	Flax-Seed
ALA	450	220			1400		
ARIZ	32	18	82				
ARK	55	620			4050		
CALIF	570	55	100				
COLO	840	500		75			
CONN	64						
DEL	160				260		
FLA	250				340		
GA	1080	165			2100		
HAW							
IDAHO	165			400			
ILL	11200	320			9200		
IND	6200	20			4400		
IOWA	13400	20			8500		
KANS	1050	4800			1730		
KY	1650	130			1520		
LA	95	290			2450		
MAINE	42						
MD	685				435		
MASS	45						
MICH	3050				1200		
MINN	7250		35	2200	5300	264	50
MISS	120	415			3300		
MO	2100	1400			5500		
MONT	90		215	2100			
NEBR	7400	2100			2600		
NEV				18			
N H	27						
N J	140				135		
N MEX	78	300					
N Y	1350						
N C	1800	75			1850		
N DAK	970		2750	5450	750	2850	400
OHIO	4150				3800		
OKLA	75	530			240		
OREG	75			70			
PA	1780				175		
R I	4						
S C	490	60			1520		
S DAK	3400	560	95	1900	1400	600	105
TENN	850	280			1900		
TEX	1680	4350			450	40	
UTAH	82			39			
VT	113						
VA	750	21			750		
WASH	160			220			
W VA	115						
WIS	4150			20	480		
WYO	112			25			
U S	80394	17249	3277	12517	67735	3754	555

UNIT IS 1000 ACRES; USDA SRS CRB 1984 CROP PRODUCTION SUMMARY

TABLE 3. (continued)

	Dry bean	Dry lima	Potato	TOTAL
ALA			13.2	2083.2
ARIZ			5.4	137.4
ARK				4725
CALIF	191	64	61.8	1041.8
COLO	195		60.8	1670.8
CONN			1.5	65.5
DEL			5.5	425.5
FLA			34.9	624.9
GA				3345
HAW				0
IDAHO	140		330	1035
ILL			2.5	20722.5
IND			5.2	10625.2
IOWA			1.7	21921.7
KANS	13			7593
KY				3300
LA			1.1	2836.1
MAINE			94	136
MD			1.6	1121.6
MASS			3.4	48.4
MICH	400		58	4708
MINN	52		83.3	15234.3
MISS				3835
MO				9000
MONT	8.5		7.5	2421
NEBR	175		10.8	12285.8
NEV			10	28
N H				27
N J			8.6	283.6
N MEX			9.3	387.3
N Y	35		40	1425
N C			17.7	3742.7
N DAK	205		136	13511
OHIO			10.7	7960.7
OKLA				845
OREG			57.5	202.5
PA			22	1977
R I			2.7	6.7
S C				2070
S DAK			15	8075
TENN			3	3033
TEX			17.9	6537.9
UTAH	9.5		6.5	137
VT			0.3	113.3
VA			16	1537
WASH	35		116	531
W VA				115
WIS			62	4712
WYO	38		3.3	178.3
US	1497	64	1336.7	188378.7

UNIT IS 1000 ACRES; USDA SRS CRB 1984 CROP PRODUCTION SUMMARY

TABLE 4. 1984 STATE AREA PLANTED TO FROST SENSITIVE VEGETABLE CROPS.

	Total fresh market	S. Corn fr	Melon fr	Lettuce fr	Tomato fr	Asparagus fr & pr	Srawberry fr & pr
ALA	9350	5500			3850		
ARIZ	54800		2200	41900			
ARK	2000				2000		300
CALIF	483300	14200	16300	152100	27900	34200	14100
COLO	20300	3500		2900			
CONN	5300	5300					
DEL							
FLA	146750	60400		15850	48800		5200
GA	3000				3000		
HAW	1010				280		
IDAHO	4700						
ILL	5300	3900				1400	
IND	3300				1400	*	
IOWA							
KANS							
KY							
LA	370				370		600
MAINE							
MD	2730				2600	*	
MASS	10290	9600			690		
MICH	59900	13300		1200	3600	20000	2800
MINN	4200					*	
MISS							
MO							
MONT							
NEBR							
NEV							
N H							
N J	25300	13000		3100	6200	2000	1100
N MEX	10100			6000			
N Y	56610	28200		4200	3100		3100
N C	6800	5000			1800		2200
N DAK							
OHIO	20200	16000		800	1500		1600
OKLA							
OREG	24300	2000				*	6800
PA	21200	16600		4600			1700
R I							
S C	4700			4700			
S DAK							
TENN	4500			4500			
TEX	71400	3400	5200	3600	4200		
UTAH	2300						
VT							
VA	6000	3500			2500		
WASH	44800	2800		1100		31000	3000
W VA							
WIS	7900			700			1600
WYO							
OTHERS*						4730	
U S	1122710	206200	23700	233450	127590	93330	44100

TABLE 4. 1984 STATE AREA PLANTED TO FROST SENSITIVE VEGETABLE CROPS. (cont)

	Total processing	Bean pr	Corn pr	Cucumber pr	Peas pr	Tomato pr	
ALA	2800	*		*			
ARIZ	*			*			
ARK	4370	4100		*			
CALIF	6675410	*		3400	*	244000	
COLO	32700			2400		1100	
CONN							
DEL	72270	*	5200	1500	11600	840	
FLA	41700	2700		5100		*	
GA	9580	*		*			
HAW							
IDAHO	159310	*	17700	*	*		
ILL	285120	*	37400		*	*	
IND	202780	3000	*		780		7900
IOWA	34030	*	*		*	*	
KANS							
KY	*			*			
LA							
MAINE	10590				*		
MD	138280	*	8700	3500	2600	3100	
MASS	*			*			
MICH	343200	18400		22000	*	8000	
MINN	790170	*	120100	*	78300		
MISS	*			*			
MO	3470	*		*			
MONT							
NEBR	*			*			
NEV							
N H							
N J	113940	8900		1100	*	4100	
N MEX	*					*	
N Y	278390	35100	29300	*	9600	*	
N C	93580	*		28700		*	
N DAK							
OHIO	445850			5000		16500	
OKLA	*	*					
OREG	568470	24600	42500	2000	36000		
PA	93470	5300	3900		*	3500	
R I							
S C	36050	*		9400		*	
S DAK	*			*			
TENN	11340	5400					
TEX	80310	*		6800		*	
UTAH	8150	*			*	*	
VT							
VA	18180	*		*		1300	
WASH	375700	1200	35600	1500	66300		
W VA	*					*	
WIS	1062650	78800	136300	8100	91000		
WYO							
OTHERS*	21160	38990	9200	7150	50770	8260	
U S	12013020	226490	445900	108430	346170	298600	

TABLE 4. (cont)

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* UNIT IS ACRES
VEGETABLES 1985 PRELIMINARY ACREAGE, YIELD, PRODUCTION & VALUE
USDA, SRS, CRB
fr = fresh market
pr = processing crops
Others = other states included in total

TABLE 5. 1982 STATE AREA PLANTED TO FROST SENSITIVE DECIDUOUS FRUITS

	Apple	Apricot Nectarine	Cherry	Grape	Peach	Pear	Plum Prune	TOTAL
ALA	1548	32	11	258	5179	145	146	7319
ARIZ	1969	359	8	3690	861	65	829	7781
ARK	2976	77	23	2475	3733	102	78	9464
CALIF	29933	50463	12453	756720	74428	30819	122394	1077210
COLO	6811	183	962	103	2422	760	153	11394
CONN	4238	8	18	111	606	345	25	5351
DEL	888			34	363			1285
FLA	253	133		644	1507	116	40	2693
GA	4656	29	9	1068	22685	148	183	28778
HAW							10	10
IDAHO	8408	111	871	250	877	268	1232	12017
ILL	9197	60	85	226	3441	100	110	13219
IND	6027	69	73	385	1123	111	55	7843
IOWA	2308	8	35	41	49	38	19	2498
KANS	2064	31	58	51	1200	55	30	3489
KY	3489	23	46	129	1279	47	30	5043
LA	61	1	1	39	1169	35	31	1337
MAINE	7633	2	18	38	25	32	17	7765
MD	7119	27	234	406	3375	129	82	11372
MASS	8457	5	21	177	447	185	26	9318
MICH	69715	305	65372	12787	10436	2286	4159	165060
MINN	4073	5	29	99	2	13	54	4275
MISS	295	34	3	239	1513	85	65	2234
MO	7218	74	73	1531	3783	107	52	12838
MONT	365	4	1304	48	1	9	19	1750
NEBR	468	5	56	13	27	4	7	580
NEV	137	12	9	9	22	7		196
N H	4100	3	10	89	80	46	6	4334
N J	7719	23	90	370	14254	89	69	22614
N MEX	3397	63	187	324	225	138	35	4369
N Y	78115	73	8544	42832	3038	3568	1049	137219
N C	18557	39	37	1628	5099	50	27	25437
N DAK	73		2			1	9	85
OHIO	14567	60	470	3067	2319	389	241	21113
OKLA	1710	98	55	87	2076	185	99	4310
OREG	11633	255	16599	2589	1837	19896	5307	58116
PA	39150	685	2696	11513	11927	1478	403	67852
R I	579			97	125	13	3	817
S C	3279	198	8	1358	40093	34	117	45087
S DAK	114	1	3		2		38	158
TENN	3517	50	74	413	2654	158	78	6944
TEX	870	323	42	943	9755	756	422	13111
UTAH	3970	244	4944	169	2186	383	131	12027
VT	4936		8	7	3	11	8	4973
VA	29972	103	138	794	4899	93	69	36068
WASH	145630	1469	14277	26728	2796	21477	2345	214722
W VA	18608	110	202	196	3623	48	33	22820
WIS	10400	7	4240	211	13	74	71	15016
WYO	41		11	1		3	4	60
U S	590541	55964	134194	874996	247561	84915	140413	2128584

Unit is acre; 1982 Census of Agriculture

TABLE 6. 1984 STATE AREA PLANTED TO CITRUS FRUITS

State	Acres
Arizona	41200
California	259592
Florida	694800
Texas	67536
U S	1063128

Unit is acre; Statistical Reporting Service

TABLE 7. PROPORTION OF NON-CONIFEROUS STATE AREA PLANTED TO FROST SENSITIVE CROPS.

State	Total area planted to frost sensitive crops	*Percent of non-coniferous area planted to frost sensitive crops
ALA	2102669	6.5
ARIZ	241181	0.3
ARK	4740834	14.2
CALIF	9537312	10.2
COLO	1735194	2.7
CONN	76151	2.5
DEL	499055	40.6
FLA	1510843	4.4
GA	3386358	9.1
HAW	1020	0.02
IDAHO	1211027	2.6
ILL	21026139	59.0
IND	10839123	47.1
IOWA	21958228	61.3
KANS	7596489	14.5
KY	3305043	13.0
LA	2837807	10.0
MAINE	154355	0.8
MD	1273982	20.3
MASS	68008	1.4
MICH	568160	1.6
MINN	16032945	32.4
MISS	3837234	12.7
MO	9016308	20.5
MONT	2422750	2.8
NEBR	12286380	25.7
NEV	28196	0.04
N H	31334	0.6
N J	445454	9.3
N MEX	401769	0.5
N Y	1897219	6.4
N C	3868517	12.4
N DAK	13511085	30.4
OHIO	8447863	32.2
OKLA	849310	2.0
OREG	853386	1.4
PA	2159522	7.5
R I	7517	1.1
S C	2155837	11.2
S DAK	8075158	16.6
TENN	3055784	11.6
TEX	6770257	4.0
UTAH	159477	0.3
VT	118273	2.0
VA	1597248	6.3
WASH	1166222	3.1
W VA	137820	0.9
WIS	5797566	16.8
WYO	178360	0.3
U.S.	204706142	11.0

TABLE 7 (cont)

*The acres planted to frost sensitive crops (Table 4), vegetables (Table 5), and fruit trees (Tables 6 & 7) were summed to obtain the total acreages of frost sensitive plants. This area on a state by state basis was divided by the area not occupied by coniferous forests (from Table 2) to obtain the estimated proportion of land per state planted to frost sensitive plants.

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